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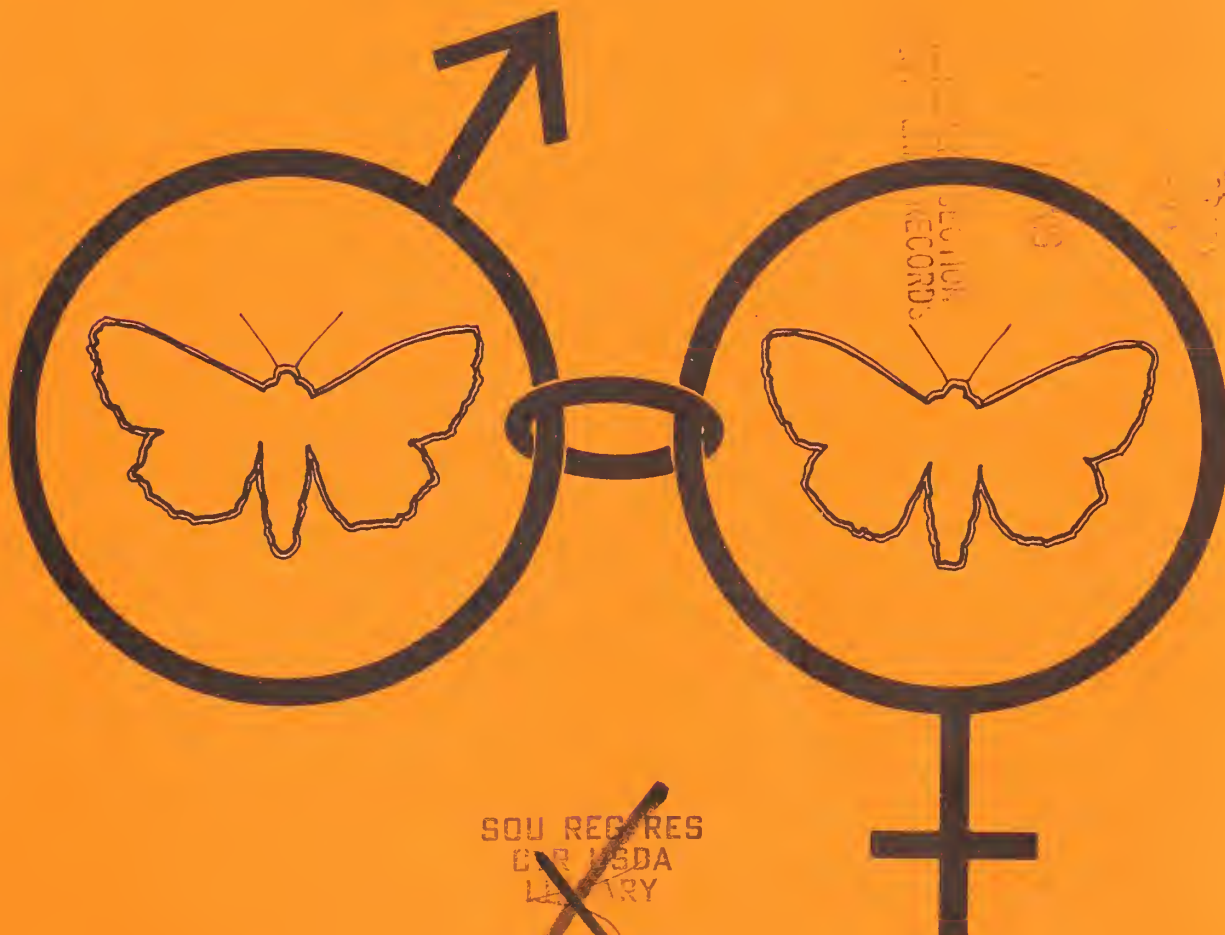


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FIRST SEMI-ANNUAL REPORT

1973

*Insect Attractants, Behavior, and Basic Biology*

*Research Laboratory*

*USDA-ARS, Southern Region, Florida-Antilles Area*

*P. O. Box 14565*

*Gainesville, Florida 32604*

In Cooperation with the

Department of Entomology and Nematology

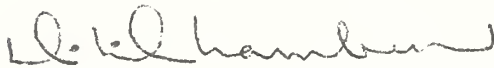
University of Florida

This progress report includes tentative results of research not sufficiently complete to justify general release. Such findings, when adequately confirmed, will be released through established channels. Therefore, this report is not intended for publication and should not be referred to in literature citations.



## PREFACE

The period from July 1972 until now has been one of growth and organization at this laboratory. Combined with the reorganization of ARS, this caused a suspension of preparation of quarterly reports, the lack of which has been felt in many ways. We plan to rectify this with these semi-annual reports. The format used herein was designed to provide multiple utility and readability. Hopefully, these benefits offset the additional bulkiness resulting from limiting each page to one report.

A handwritten signature in dark ink, appearing to read "D. L. Chambers", with a stylized, cursive script.

Derrell L. Chambers  
Director





Growth and Development

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Juvenile Hormone Binding by Haemolymph  
Proteins in the Indian Meal Moth

S. M. Ferkovich, D. L. Silhacek, and R. R. Rutter

Objectives: To understand the transport of juvenile hormone (JH) in the haemolymph from its site of synthesis to target tissues. Others showed that lipoproteins may act as hormone carriers; their isolation should facilitate investigations of substances (hormone analogs, etc.) that interfere with their capacity to carry or unload JH at its site of utilization. We, therefore, are studying the binding affinity of JH for select blood proteins and the significance of such proteins in hormone transport in the Indian meal moth, Plodia interpunctella.

Methods: Haemolymph from 4 mg and 21 mg fifth-instar larvae was incubated with  $^3\text{H}$ -JH and fractionated on G-200 columns and the molecular weights of the resolved protein peaks estimated. The proteins were analyzed by disc gel electrophoresis. JH and its metabolites were analyzed by thin layer chromatography.

Results: The elution pattern and associated radioactivity for haemolymph from 4 mg larvae is shown in Figure 1. A radioactive peak was associated with the first peak, which contained proteins with molecular weights estimated at 155,000 and 203,000. Over two-thirds (76%) of the count was still JH; the remainder was associated with dihydroxy methyl ester (16%), epoxy acid (6%) and dihydroxy acid (2%). The second radioactive peak did not coincide with any apparent protein peak and was primarily metabolized JH; only 6% was JH, and 5%, 39% and 50% was metabolized to the dihydroxy methyl ester, epoxy acid and dihydroxy acid, respectively. Most activity in the first peak proteins was associated with six slow moving bands, two of which were lipoproteins. When haemolymph from mature 21-mg larvae was incubated with  $^3\text{H}$ -JH and fractionated, only one radioactive peak (which represented metabolized JH) eluted between the two protein peaks, indicating that deactivating enzymes in the haemolymph are involved in regulation of hormone titre. Preliminary studies to demonstrate the juvenilizing effects of the JH-bound proteins by testing their capacity to inhibit cuticle deposition in wing imaginal discs from the Indian meal moth in vitro (in cooperation with H. Oberlander) were unsuccessful.

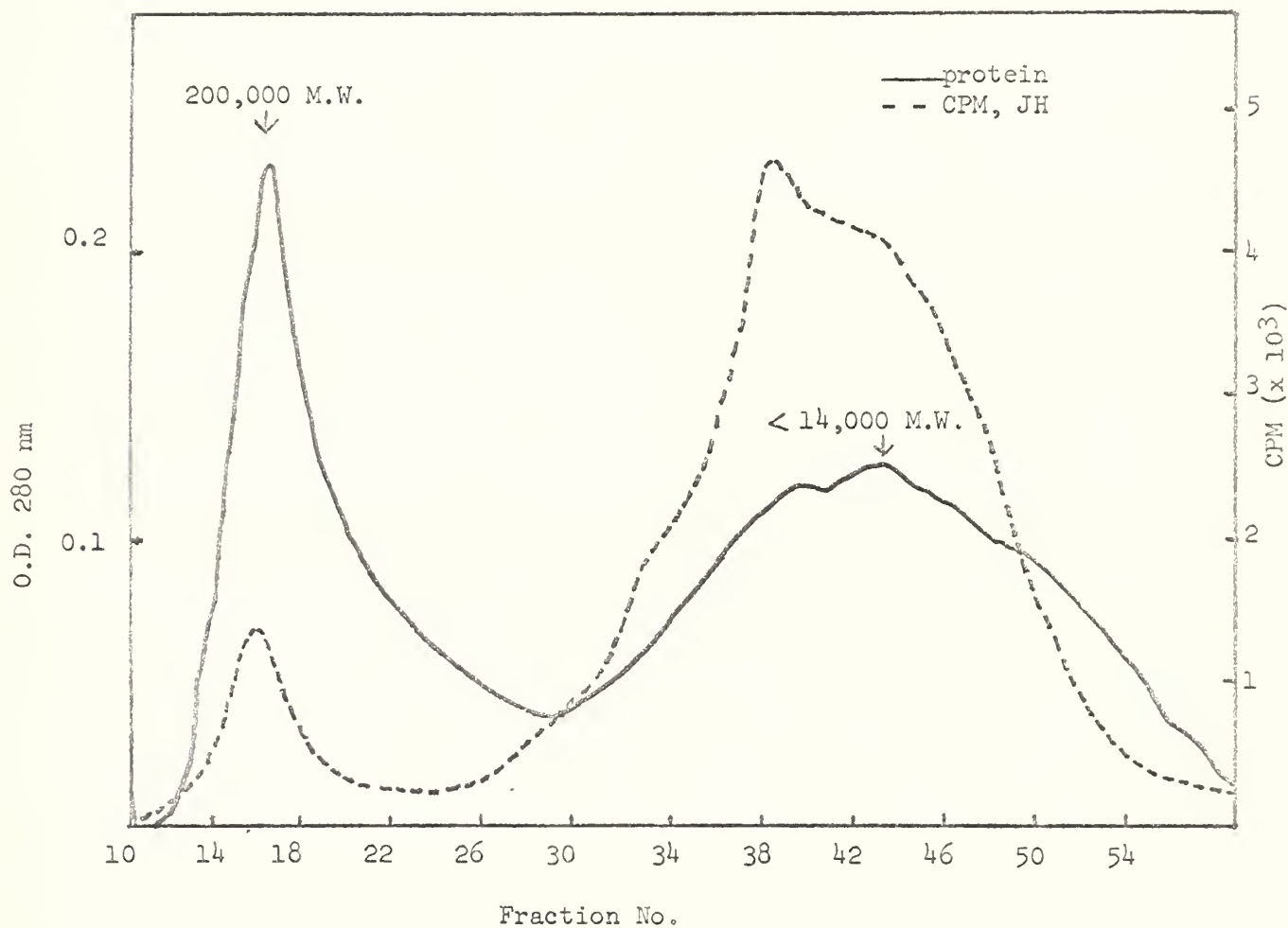
A bioassay for JH activity based on certain morphological characters of Tenebrio molitor larvae was tested and found to have adequate sensitivity for determination of JH content in the haemolymph of the Indian meal moth.

Plans: A manuscript is in preparation. Other objectives will be to: 1) demonstrate that the haemolymph proteins which bind JH possess juvenilizing activity both in vitro and in vivo, 2) determine the JH titre of the haemolymph during larval and pupal development and 3) correlate the JH level with the activity of JH degradative enzymes.





Figure 1.--Elution pattern from a Sephadex G-200 column of  $^3\text{H}$ -label from  $^3\text{H}$ -juvenile hormone incubated with haemolymph from 4 mg fifth-instar larvae of the Indian meal moth for 15 min.



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Juvenile Hormone Binding in Subcellular  
Components of the Insect Using a  
Standard Laboratory Spectrophotometer

S. M. Ferkovich and R. R. Rutter

Objectives: To establish the site of juvenile hormone (JH) action at the cellular and subcellular levels and isolate and identify receptor molecules responsible for binding the hormone. Such studies could yield a highly selective and rapid assay system for developing more effective hormone and antihormone compounds. In this study, we investigated the binding of JH by subcellular fractions of the Indian meal moth (Plodia interpunctella) using a spectrophotometer designed for routine application. Such a technique might obviate the need for radiolabelled compounds.

Methods: Whole fifth-instar Indian meal moth larvae were fractionated using a discontinuous Ficoll gradient centrifugation procedure. Electron micrographs were made of the fractions for visual identification of the cell organelles. Absorption measurements were made with a Gilford Model 2000 single beam recording photometer coupled to a Beckman D.U. monochromator. The absorbance control system was used to attain a zero baseline by negating the absorbance of the sample and JH. Generally, the change in difference absorption at 280 nm induced upon mixing Cecropia JH hormone ( $1 \times 10^{-4}M$ ) to the particulate fractions (20  $\mu g$  protein/ml) was recorded.

Results: Table 1 shows the degree of negative absorption change induced upon addition of the hormone to the various particulate fractions. Treatment with sulfhydryl inhibitors (NEM, P-CBA) reduced, and heat (64°C) destroyed activity of the fractions, indicating that the observed absorbance change with JH involved protein(s). The greatest interaction occurred with the particulate portion of the homogenate, especially in the fraction containing fragmented cell membranes ( $F_1$ ). However, it is not known whether the change was due to enzymatic or nonenzymatic binding (complexing). These preliminary results suggest that the technique can be effectively used to measure binding of JH in cell components in insects.

Plans: A manuscript is in preparation.



Table 1.--Negative change in absorbance of fractions of the Indian meal moth at 280 nm induced upon addition to 20  $\mu$ g protein/ml of the indicated fraction.

Sucrose layer	Fraction	Predominant component	$-\Delta A/5 \text{ min}$
0.8M	F <sub>1</sub>	Cell membrane fragments	0.104
1.0	F <sub>2</sub>	Small mitochondria, membrane fragment studded with ribosomes	0.085
1.2	F <sub>3</sub>	Large mitochondria	0.074
1.5	F <sub>4</sub>	Large mitochondria, lysosomes	0.059
1.8	F <sub>5</sub>	Nuclear material	0.053
pellet	F <sub>6</sub>	Tissue fragments	0.037
	Supernatant	Supernatant (with microsomes)	0.028
		Microsomes	0.05



Effect of Juvenile Hormone and Its Mimics on Mitochondrial  
Metabolism in the Indian Meal Moth,  
Plodia interpunctella

D. L. Silhacek

Objective: In studies on the effects of hormones on intermediary metabolism of Indian meal moths, we found that in vitro juvenile hormone (JH) inhibits some mitochondrial oxidations while stimulating others. These results suggested that the JH effects on mitochondrial metabolism might provide valid criteria for evaluating potentially active JH-mimics. The purpose of the present experiments was to determine the feasibility of developing such a method.

Methods: Mitochondria were isolated from Indian meal moth larvae by differential centrifugation. Mitochondrial oxidative activities with succinate and pyruvate-malate as substrates were determined with a vibrating platinum electrode. Initial experiments were conducted with mitochondria isolated from larvae of different known ages. Subsequent experiments were conducted with mitochondria isolated from newly molted last-instar larvae.

Results: The results indicated that late 4th- and early 5th-instar larvae yielded mitochondria which oxidized succinate at twice the normal rate when JH was added. However, the stimulatory effect of JH on succinate oxidation was gradually lost as larvae grew to maturity. On the other hand, JH inhibition of pyruvate-malate oxidation was unaffected by larval age.

Initial experiments with several different JH mimics indicated that their juvenilizing activity in vivo did not parallel their effectiveness in the mitochondrial assay. Certain structural features of the molecule appeared to be necessary in producing the mitochondrial effects in vitro but were not required in producing the morphological effects in vivo.

Plans: Studies will continue on determining what molecular structural characteristics are needed for affecting mitochondrial metabolism in vitro. Additional experiments will determine if JH treatment of larvae will affect their mitochondrial metabolism.





Influence of Juvenile Hormone on Reproductive  
Behavior of the Indian Meal Moth

L. L. Sower, H. Oberlander and D. L. Silhácěk

Objective: To determine whether juvenile hormone treatment of the Indian meal moth, Plodia interpunctella (Hübner), prior to metamorphosis would affect the reproductive behavior of insects which emerge as "normal" adults.

Methods: Larvae of the Indian meal moth were reared on juvenile hormone-(ENT 33972a) treated diet for varying periods of time. We tested the mating frequency, pheromone content, and attractancy to pheromone of control and hormone-treated insects.

Results: Treatment of larvae with a concentration of juvenile hormone (25ppm) which permitted most of the larvae to complete metamorphosis greatly reduced mating frequency and female calling behavior. The female sex pheromone content was not affected. Even if treatment was stopped well before the end of the last larval instar, mating frequency in the resulting adults was reduced.

Plans: We are determining the period in larval life most sensitive to juvenile hormone treatment as measured by mating inhibition in the adult. We will investigate the mode of action of juvenile hormone in causing this delayed behavioral effect.



Exposure of Stored-Product Coleoptera to  
Hormone-treated Culture Medium

F. O. Marzke and W. G. Sercey

Objective: To determine the effect on development of exposure of the cigarette beetle, (Lasioderma serricorne) or red flour beetle, (Tribolium castaneum) to culture medium treated with juvenile hormone-mimicking compounds.

Methods: Cigarette beetle and red flour beetle culture medium (flour, corn meal, and brewers yeast) was treated with juvenile hormone-mimicking compounds at 0.5-250 ppm. Eggs, last instar larvae or adults were placed in the treated medium and the subsequent development of the insects observed.

Results: Zoecon 515 continued to be the most effective for preventing development; concentrations as low as 1 ppm prevented adult emergence. When cigarette beetle females were allowed to oviposit on medium treated with as high as 250 ppm of Zoecon 515, little effect was noted on the development of the offspring until the last instar or pupal stage. If adults were allowed to oviposit in the treated medium for 3 days and then transferred to fresh untreated medium, prior exposure of the adults to the hormone appeared to have little effect on the development of progeny in the untreated medium.

Last instar red flour beetle and cigarette beetle larvae were exposed for 1 or 2 weeks or 1, 2, 3, or 4 months in medium treated with Zoecon 515 or Hoffmann LaRoche 33972a at 0.5, 5, 50 or 250 ppm and then transferred to clean medium, if still alive. One week in treated medium did not prevent adult emergence; 1 month prevented emergence of the red flour beetle, but a few cigarette beetle adults emerged even after several months' exposure.

The age of eggs of the cigarette beetle placed on medium treated with Zoecon 515 or Hoffmann LaRoche 33972a, appeared unrelated to further development of the insect.

JH 25 was about as effective as was Zoecon 515 in preventing further development of last instar larvae of the red flour beetle placed in treated medium, but had little effect on the cigarette beetle.

Plans: Studies will be expanded on the effects of aging on Zoecon 515. The relationship of the concentration of the hormone in the medium to the possible sterilization of red flour beetles and cigarette beetles will be investigated.



Exposure of Stored-Product Coleoptera to  
Hormone-treated Papers

F. O. Marzke and W. G. Sercey

Objectives: To determine whether confinement to surfaces treated with juvenile hormone-mimicking compounds would prevent development of the cigarette beetle, (Lasioderma serricorne) or red flour beetle, (Tribolium castaneum).

Methods: Whatman No. 1 filter papers were treated with an acetone solution of juvenile hormone-mimicking compounds Zoecon 515, Hoffmann-LaRoche 33972a, Stauffer 20458 or JH 25 at 10, 30, 100 or 300  $\mu\text{g}/\text{cm}^2$ . Five- to 7-day old mated and unmated cigarette beetle adults and mated red flour beetle adults were confined to the treated surfaces for 24 hours, and then transferred to vials containing 2 grams of untreated culture medium. The number of eggs and larvae and adult emergence were then determined. Mated cigarette beetle females were also exposed to paper treated with Zoecon 515 for 1/2 and 1 hour.

Results: No offspring were observed when mated female cigarette beetle adults were confined for 24 hours to paper treated with Zoecon 515 at 100 or 300  $\mu\text{g}/\text{cm}^2$ , apparently as a result of the insects dying as embryos and not from failure of the adults to oviposit. The other juvenile hormones had little effect on the  $F_1$  generation. Exposure for an hour or less to Zoecon 515 at 300  $\mu\text{g}/\text{cm}^2$ , produced a slight reduction in the  $F_1$  generation. There was little effect on the  $F_1$  generation of the red flour beetle when the mated females were exposed to paper treated with any of the juvenile hormone-mimicking compounds.

In general, when unmated female cigarette beetles were confined for 24 hours to paper treated with Zoecon 515 at 300  $\mu\text{g}/\text{cm}^2$  and then mated, results were similar to those obtained with females mated before exposure to the treatment. However, in a couple of the vials in which the females oviposited a few larvae were observed.

Plans: Research will be continued using other periods of exposure to the papers treated with Zoecon 515 at 300  $\mu\text{g}/\text{cm}^2$ . The length of time the treated papers remain effective will be investigated. Studies will be conducted to determine whether exposure of the adults to concentrations of juvenile hormone-mimicking compounds which do not prevent egg hatch will have a delayed effect on the last instar larvae or pupae.



Interactions Between Beta-ecdysone and Fat Body In vitroH. Oberlander, A. B. Dutkowski<sup>1/</sup> and S. M. Ferkovich

Objectives: In our study of interactions between ecdysone and tissue factors in the insect we have determined that a factor from the larval fat body stimulates cuticle deposition in Lepidopteran imaginal discs which are incubated in vitro with beta-ecdysone. The focus of the project at this time is on the mode of action of the fat body factor.

Methods: Mature final instar larvae of the Indian meal moth, Plodia interpunctella (Hübner), were used as donors of fat body and wing discs. The tissues were cultured in plastic petri dishes in a modified Grace's tissue culture medium. The response of the discs to beta-ecdysone was tested in plain medium and in fat body conditioned medium. An extensive series of sequential incubations was employed to reveal the mode of action of the fat body factor.

Results: Our experiments show that the same concentration of beta-ecdysone (2 µg/ml) which stimulates cuticle deposition under one set of conditions inhibits it under other circumstances. We found three ways in which beta-ecdysone stimulated cuticle deposition in vitro in imaginal discs: (1) If the discs were incubated with beta-ecdysone (0.5-50.0 µg/ml) in the presence of fat body factor, (2) if the discs were incubated with 0.2 µg/ml beta-ecdysone in plain medium, or (3) if the discs were incubated with beta-ecdysone (0.5-5.0 µg/ml) for 24 hours and then transferred to hormone-free medium. We believe that the fat body factor may inactivate beta-ecdysone once the discs have been stimulated, thereby creating a pulse treatment of hormone.

Plans: We are investigating the chemical nature of the fat body factor. This will assist us in our investigation of its mode of action and possible use as an intervening substance in insect development.

Publication: A. B. Dutkowski and H. Oberlander. The influence of larval fat body on wing disk development in vitro. Journal of Insect Physiol. In press.

1/ Visiting scientist from M. Nencki Institute of Experimental Biology, Warsaw, Poland.





In Vitro Bioassay of Insect Hormones

H. Oberlander

Objective: To develop sensitive and fast bioassay procedures for insect hormones and hormone mimics. Compounds which may have ecdysone, anti-ecdysone or juvenile hormone activity will be evaluated by an in vitro bioassay system as an adjunct to standard in vivo tests.

Methods: Wing discs of the Indian meal moth, Plodia interpunctella (Hübner), are incubated in vitro in modified Grace's tissue culture medium with the candidate hormonal agent. The ability of the compound to stimulate evagination, tracheole migration or cuticle deposition is noted.

Results: A variety of ecdysone and analogues have been tested. To date only beta-ecdysone and deoxy-crustecdysone stimulated cuticle deposition. Our studies emphasize the importance of the 20-hydroxyl position. Podecdysone-A, 22-isoecdysone, 5- $\beta$ -ketodiol, and 22-25 bisdeoxyecdysone had little or no effect on the disks.

Plans: Structure-activity and inhibitory relationships amongst ecdysone analogues will be explored further. The effects of juvenile hormone analogues on ecdysone-induced morphogenesis in vitro will be investigated.



Investigation of Hormones and Enzymes of Cuticular  
Tanning in Cabbage Loopers,  
Trichoplusia ni

S. L. Carlisle, D. L. Silhacek and N. C. Leppla

Objectives: Insects displaying variations in cuticular tanning that differ markedly from the normally tanned adults, are potentially useful as markers for field releases and as tools for basic biological research. Our current research involves the identification of hormones, enzymes, and substrates participating in cuticular tanning, and of their sites and modes of action. Of particular interest are the effects of various hormones upon tanning and the potential use of these hormones and specific inhibitors to produce marked variants.

Methods: We are testing soluble forms of tyrosine and its metabolites, the primary tanning substrates, for use in bioassays and biochemical determinations. Insect hemocytes appear to be the active sites of tyrosine metabolism, so we are developing techniques for the collection and isolation of large quantities of intact hemocytes. The influences of various hormones, enzymes, and inhibitors are monitored by measuring rates of oxygen utilization by isolated hemocytes treated with various concentrations of the test compounds.

Results: Initiation or acceleration of tanning metabolism has resulted from treatment with certain hormones. However, techniques have not been sufficiently refined as yet to provide quantifiable data.

Plans: We will pursue current investigations to increase our understanding of the basic biological processes of cuticular tanning in the cabbage looper and other test insects. Concurrently, methods for disrupting normal tanning metabolism without interfering with normal behavior will also be studied.



Uptake and Metabolism of  $\alpha$ - and  $\beta$ -Ecdysone by  
Cultured Wing Discs of Plodia  
interpunctella

D. L. Silhacek and H. Oberlander

Objective: Previous studies have provided considerable insight into the physiological mechanism of ecdysone action on cultured wing discs. However, for meaningful interpretation of these findings, it was essential to determine the metabolic fate of ecdysone when added to the tissue culture medium. The purpose of this study was to determine whether ecdysone is converted to other active (or inactive) molecules during the tissue incubations and to measure the extent of ecdysone binding to the wing discs.

Methods: Wing discs were dissected from Indian meal moth larvae and cultured by established methods. Procedures for the quantitative extraction, separation, and measurement of ecdysone and its metabolites were investigated using isotopically labelled hormone.

Results: Satisfactory separation of  $\alpha$ - and  $\beta$ -ecdysone by thin layer chromatography was achieved. The developed chromatogram was fractionated by sequentially removing zones of the adsorbent. By using tritiated ecdysone, quantitative estimates of compounds in the various zones were accomplished. Quantitative extraction of ecdysone from tissue culture medium has not proven satisfactory although a variety of methods have been tried.

Plans: A method for the quantitative recovery of ecdysone and its metabolites from tissue culture medium will be developed. Experiments investigating the metabolism and distribution of tritiated ecdysone during wing disc incubation will be conducted.



# Hormonal Control of Chitin Synthesis In vitro

H. Oberlander and C. E. Leach

Objective: To investigate the mode of action of ecdysone and juvenile hormone on the initiation and inhibition of metamorphosis, we have developed a model system. Ecdysone stimulates and juvenile hormone inhibits cuticle deposition in imaginal discs in vitro. We are focusing on the action of these hormones on the biosynthesis of chitin.

Methods: Wing discs of the Indian meal moth, Plodia interpunctella (Hübner), are cultured in vitro in a modified Grace's medium. The incorporation of D-glucosamine-6- $H^3$  into the discs is evaluated with radioautographic and scintillation counting methods.

Results: Both alpha-ecdysone and beta-ecdysone stimulate uptake of D-glucosamine-6- $H^3$  by the discs. However, only beta-ecdysone stimulates incorporation of this isotope into chitin. The incorporation of isotope precedes the general appearance of tanned cuticle by about one week. The addition of glucosamine or N-acetyl glucosamine to the tissue culture medium has no effect on cuticle deposition in the discs.

Plans: We will investigate the uptake and incorporation of other amino sugar isotopes to determine the specificity of ecdysone's action. We will then study the inhibition of uptake and/or incorporation of these chitin precursors by juvenile hormone.





Reproductive Biology of the Cigarette Beetle,  
Lasioderma serricorne (Fabr.)

J. A. Coffelt

Objectives: To determine the role(s) and/or interactions of various environmental, physiological, and behavioral factors which influence the reproductive behavior of the cigarette beetle. The studies reported herein had the specific objective of characterizing the influence of the initial mating upon subsequent oviposition and remating behavior by the female.

Methods: Single-pair matings of sexually mature beetles were made to determine the significance of the duration of mating upon subsequent female oviposition and remating behavior. Pairs in copulo were manually separated 15, 25, and 45 min after the onset of mating. Two groups served as controls; the first in which no mating occurred, and a group in which mating was not interrupted ( $\bar{x}$  = 80.1 min). Subsequently, females were either dissected within 5 min after mating, placed upon a suitable oviposition site for 1-4 days, or held in empty containers for 1, 2, 4, 24, 48 or 72 hr and then placed with a second male to determine if remating would occur. The remating behavior of mated parous and nulliparous females was compared by recording the proportion of females of each that remated 1, 2, 3, 4, 5, 6, 7, 10, 15, and 20 days after the first mating.

Results: The relationship between the duration of the first mating and remating behavior within the following 4 hr is summarized in Table 1. The influence upon the remating behavior after 24, 48 and 72 hr was similar to that recorded in Table 1; that is, females that had mated for 45 min did not remate, nor did those that had completed mating without being manually separated. Remating by females that had initially mated for 15 min was near 100% after 24 hr. Remating by females initially mated for 30 min was ca. 50% after 72 hr. The remating behavior of parous and nulliparous mated females is compared in Fig. 1. Unmated females were not observed to oviposit. The data suggest that insemination was required to stimulate oviposition and that a secretion from at least 1 pair of male accessory glands had at least a temporary effect upon remating among mated-uninseminated females. Further, oviposition appeared to remove the inhibition to remating within 1-2 days.

Plans: The significance of multiple matings by individual females will be determined. The contribution, if any, of multiple matings to fecundity increases will be determined. The role of spermatozoa received in second matings will be studied by using genetic markers. Attempts will be made to correlate willingness to remate with pheromone content among both parous and nulliparous females.



Table 1.--Relationship between the duration of the first mating, transfer of male components, and remating by cigarette beetle females.

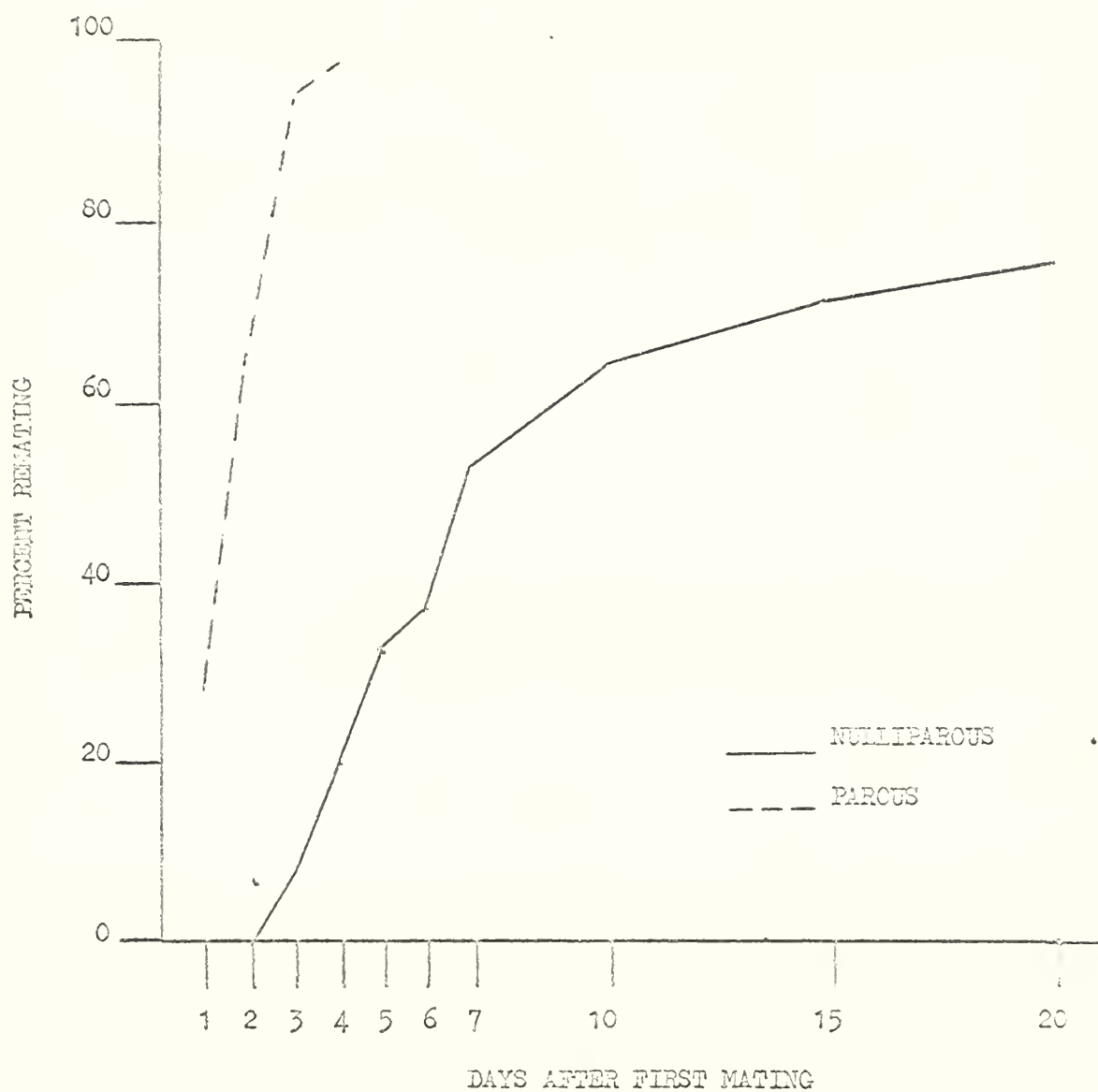
Duration of first mating (min)	N	Cumulative % remating w/in:			Percent	
		1 hr	2 hr	4 hr	Male gland secretion transfer	Insemination
0	40	97.5	100	100	0	0
15 <sup>a</sup>	41	14.6	26.8	43.9	0	0
30 <sup>a</sup>	39	0	2.5	2.5	84.6	0
45 <sup>a</sup>	40	0	0	0	100	100
80.1 $\pm$ 3.6 <sup>b</sup>	40	0	0	0	100	100

<sup>a</sup> pairs separated manually

<sup>b</sup> mean ( $\pm$  se) of undisturbed matings



Figure 1.--Comparison of the remating behavior of parous and nulliparous cigarette beetle females.





Laboratory Culture of Plum Curculios,  
Conotrachelus nenuphar (Herbst)

A. J. Hill and C. Calkins

Objective: To rear continuous generations of plum curculios in the laboratory with a minimum of effort.

Methods: Adults were procured at the Southeastern Fruit and Tree Nut Research Station at Byron, Ga., in September 1972. The insects are reared on thinning apples throughout their life. Efforts were made to reduce the labor necessary to maintain the colony.

Results: The regular laboratory plum curculio colony is being maintained at a level that produces ca. 125 larvae per day, which is more than adequate for our present daily usage, yet it provides an adequate reservoir to increase the production at least 10-fold within 25 days. Maintenance of this colony at its present level of efficiency requires about 4 hours of a technician's time and about 1/2 case of apples per week. Seven generations have been completed at this time. The length of the life cycle is 15-20 days from egg to mature larvae, 28-32 days from pupa to adult, and 5 days preoviposition period for the adult. Adult emergence from the pupal stage normally exceeds 80%. There are 4 separate strains of field collected plum curculios being maintained in the laboratory. These were initiated by collecting fruit from Monticello, Fla., (peaches), Madison, Fla., (nectarines), Gainesville, Fla., (wild plums) and Byron, Ga., (plums).

Plans: The laboratory colony is being maintained to serve as a source of supply of virgin adults for circadian rhythm, sex pheromone, and reproductive behavior studies and as the source for adults used in genetic crosses. Detailed life history and life table information is being secured using these insects. This colony will be maintained indefinitely at the level necessary for our needs. The wild populations will be maintained for life history and life table data, as well as for genetic identification and characterization by electrophoresis.





Culturing Lepidoptera: The Carpenterworm,  
Prionoxystus robiniae

N. C. Leppla, R. E. Doolittle, and J. D. Solomon<sup>1/</sup>

Objectives: To develop more efficient rearing methodology and provide research material for cooperating scientists.

Methods: During March 1973, field-collected female carpenterworm moths, Prionoxystus robiniae (Peck), were held for oviposition at the Southern Forest Experiment Station, Stoneville, Miss. Approximately 1000 of the resulting eggs, deposited at  $25 \pm 2^\circ\text{C}$  and  $60 \pm 15\%$  RH under an 8-hr photoperiod, were shipped to the Insect Attractants, Behavior and Basic Biology Laboratory, Gainesville, Fla., and held in the dark at  $27 \pm 2^\circ\text{C}$  and  $95 \pm 5\%$  RH. Larvae, which began to emerge within 10 days, were used to infest combinations of 21 diets in 12 different types of containers. Two larvae were placed in each container and transferred to a fresh environment at least weekly because of fungal contamination (Aspergillus niger and A. flavus) and bacterial decomposition of the diets. Since microorganism inhibitors were inadequate and excess moisture appeared to enhance the growth of toxic contaminants, the diet was modified, formed into 4 cm x 7 cm diam. "cakes" and baked at  $60^\circ\text{C}$  for 5 hr. Subsequently, less than 10% of the developing larvae became infected or required transfers during any week.

Results: The larval medium now includes pinto beans and alphacel in place of Nuttall oak sawdust. The water binding qualities were enhanced and a more coherent texture was provided by substituting HWG for agar and adding casein. The formulation was balanced, and fortified with cholesterol and soybean oil.

Plans: Larvae will be placed in a  $5 \pm 2^\circ\text{C}$  environment after the 90-day development period. In an attempt to terminate dormancy and initiate pupation, 15 larvae/week (after 1 month) will be transferred back to the incubator at  $27 \pm 2^\circ\text{C}$  and  $50 \pm 5\%$  RH with a 14-hr photoperiod.

The study will not be expanded and should be concluded during FY 1974.

<sup>1/</sup> J. D. Solomon, Forest Service Insect Research Laboratory, P.O. Box 227, Stoneville, Mississippi 38776



Culturing the Lovebug, Plecia nearctica,  
Under Semi-artificial Conditions<sup>1/</sup>

N. C. Leppla, J. J. Whitesell, L. C. Kuitert, and J. L. Gillmore

Objectives: To rear lovebugs by duplicating natural conditions in the laboratory and conducting basic biological studies. The project is divided into the following 3 phases: 1) development of adult cages which will allow mating and provide suitable oviposition sites (these containers must also provide for larval development), 2) determination of the optimum environmental requirements for the rearing situation, and 3) simplification of procedures and adaptation of techniques currently available for other species which feed on decaying vegetation.

Methods: Adult lovebugs, Plecia nearctica Hardy, were field-collected during May 1973 at Paynes Prairie near Gainesville, Fla. Fifty pairs were placed in each of 4 isocages (5.4 liter polyethylene box with a porous paper lid) containing a mixture of straw and sandy compost. These cages were incubated for 30 days at  $27 \pm 2^\circ\text{C}$  and  $85 \pm 5\%$  RH with a 14-hr photoperiod, and 5% sucrose solution containing 5cc of honey was provided in two 125 cc plastic feeder bottles. After 30 days the compost was carefully inspected for developing larvae. These masses of 10-30 insects were each transferred to an 8 oz plastic cup of compost covered with straw.

Results: Additional moisture has been added weekly and a sizable colony of larvae has been reared.

Plans: The study will be continued through one generation (adult parents -  $F_1$  eggs) and the larval medium will be further refined. This should be concluded during FY 1974.

<sup>1/</sup> A project of the Department of Entomology and Nematology, University of Florida, N. Leppla cooperating.



Laboratory Culture of the Peachtree Borer,  
Sanninoidea exitiosa (Say)

A. J. Hill and C. Calkins

Objective: To initiate a colony of peachtree borers for continuous laboratory rearing to serve as a source of virgin females for reproductive behavior and movement studies.

Methods: Newly hatched larvae were reared on green thinning apples using methods developed at Byron, Ga.

Results: Larvae and pupae were collected from an unsprayed peach orchard in September 1972, at Monticello, Fla. Larvae were placed in sawdust until pupation occurred. Pupae were separated from the sawdust and placed in depressions in damp sand. Several adults emerged, but over such a long period that it was difficult to have males and females available at the same time. Mating was achieved in small cages, oviposition occurred in small paper sacks, and most of the eggs hatched. Larvae developed over a 3 month period which necessitated changing apples 3 times. Several larvae pupated and 2 adults emerged but died before mating could occur.

Plans: This project has been terminated.



Culturing Lepidoptera: Calcium Alginate  
vs HWG in Cabbage Looper Diet

N. R. Spencer and N. C. Leppla

Objective: To successfully adapt alginates as gelling agents in artificial diets for Lepidoptera.

Methods: Calcium alginate does not require cooking and allows the medium to remain viscous for comparatively long periods, therefore, it would be advantageous to substitute the material for agar or HWG in cabbage looper diet. A cooked diet containing HWG and an uncooked medium with calcium alginate substituted for HWG were formulated and balanced. The pinto bean diet was modified by adding water, buffering with calcium phosphate, and high-speed blending. Thus, identical nutrients with different physical qualities may be tested with 4-6 generations of cabbage looper larvae.

Results: A "cold" diet containing the substituted alginate has been formulated and is currently being tested. Insects reared on the alginate medium will be compared with those from the HWG substrate in terms of larval life history, adult longevity, and mortality.

Plans: The study will be conducted until a medium comparable to the standard has been formulated. This should be concluded during FY 1974.





## Micro Techniques and Analytical Methods

R. R. Heath, J. H. Tumlinson, &amp; R. E. Doolittle

Objectives: To develop analytical techniques enabling elucidation of the structure of compounds in microgram quantities, thus, requiring fewer insects.

Methods: Samples are collected in glass capillary tubes from the gas chromatograph and examined by spectroscopy. In the NMR, tube design, solvent purity, high sensitivity probes, and various computer-instrument manipulations have been investigated to obtain maximum sensitivity. Infrared microcells and beam condensers have been tested. In the relatively new field of chemical ionization (CI) mass spectroscopy, different reagent gases and computer-instrument manipulation have been investigated to improve sensitivity.

Unsaturated hydrocarbons, acetates, and other compounds have been epoxidized using m-chloroperoxy benzoic acid. The epoxides have been examined by CI mass spectroscopy using methane and isobutane reagent gases, and by NMR and IR at the microgram level.

Results: Good NMR spectra can be obtained with 2  $\mu$ g of material, IR with less than 1  $\mu$ g, and mass spectrometer analysis with only 10 ng. This is 50- to 100-fold greater sensitivity than was possible only 1-2 years ago, thus, requiring considerably fewer insects to isolate and identify a pheromone. Also, CI and derivatization techniques provide more information about the structure of a molecule, including location and configuration of double bonds - - information particularly useful in certain doubly unsaturated acetates.

Plans: The micro techniques will be refined to make them more routine. Other types of compounds will be epoxidized and examined spectroscopically. New reagent gases will be experimented with in CI mass spectroscopy. Also, we plan to adapt high-speed, high resolution liquid chromatography to our needs since we feel this will conserve material by requiring fewer and milder purification procedures.

Publications: Application of chemical ionization mass spectroscopy to the determination of epoxide position in aliphatic chains. J. H. Tumlinson, R. R. Heath, and R. E. Doolittle. Submitted to NTE.



Synthesis of Sex Pheromones and Related Mono- and  
Multiple-unsaturated Acetates

R. E. Doolittle

Objectives: The synthesis of pure samples of known pheromones and other compounds of potential biological interest. These compounds are to be kept as standards in a "library." They will be useful for exploring new micro-analytical techniques, and as standards for gas-liquid, high pressure, and thin layer chromatography. These materials would also be tested as potential attractants, synergists and inhibitors with insects presently and later under study at the laboratory.

Methods: Standard synthetic methods as well as innovations and new methods have been used in the preparation of these materials. Nuclear magnetic resonance, mass spectrometric infrared and ultraviolet spectrophotometric measurements have been made on these compounds to insure their identity and purity. Gas liquid, thin layer and column chromatographic methods of analysis have been used to insure their identity and purity.

Results: The following compounds have been synthesized and examined: (E)-3-tetradecen-1-ol acetate, (E)-4-tetradecen-1-ol acetate, (E)-5-tetradecen-1-ol acetate. In addition, the acetylenic acetate precursors for the olefines are on hand.

Plans: Various mono- and multiple-unsaturated acetates and other closely related compounds will be synthesized on a continuing basis.



A Pheromone of the Tobacco Budworm,  
Heliothis virescens

J. H. Tumlinson, D. Hendricks<sup>1/</sup>, E. R. Mitchell  
and M. M. Brennan

Objectives: Isolation, identification, and synthesis of the pheromone produced by the female tobacco budworm, and incorporation of this pheromone into a survey and/or control program for this pest.

Methods: Virgin laboratory reared females, held on a reverse photoperiod, are collected when they are in the "calling" position. The whole female is rinsed with ether, the ether rinse is concentrated by evaporation, and the concentrate is chromatographed on Poragel 60A (37-75  $\mu$ ) eluted with hexane. The active fractions from this column are further purified by GLC on OV-1, Carbowax 20M, and Hi Eff IBP columns. All steps in the isolation are monitored by field cage bioassays. A sample of each fraction (about 50 FE in 0.5 ml solvent) is poured onto a piece of filter paper suspended in the middle of a cylindrical electric trap. Seven of these traps are placed in a cage containing hundreds to thousands of released, laboratory reared males. The traps are baited at night to coincide with the insects' activity period.

Results: The active pheromone has been considerably purified by the chromatographic procedures outlined above. The purified natural material has captured over 400 males in one trap in a single night. Additionally, in an early test, several wild males were captured in electrical traps baited with crude female rinse and placed alongside a tobacco field.

Plans: The pheromone will be isolated in pure form by GLC, identified by spectroscopic and micro-degradative techniques, and synthesized. The synthetic pheromone will be tested in field cages and in the field on wild males. Later it will be incorporated in trapping and control experiments.

<sup>1/</sup> D. E. Hendricks, Research Entomologist, Cotton Insects Research Laboratory, P. O. Box 1033, Old Fort Brown, Brownsville, Texas 78520



A Pheromone of the Lesser Peachtree Borer,  
Synanthedon pictipes (Grote & Robinson)

J. H. Tumlinson, C. E. Yonce<sup>1/</sup>, R. E. Doolittle,  
C. R. Gentry<sup>1/</sup>, and E. R. Mitchell

Objectives: As part of a program to develop an integrated pest management system for peach insects, the sex pheromone produced by the female lesser peachtree borer will be isolated, identified, synthesized, and field tested.

Methods: Ovipositors from 1- and 2-day old females were clipped and extracted with methylene chloride. The extract was concentrated by evaporation and chromatographed on a Bio-Sil HA column eluted with pentane and ether. The active fractions collected from this column were further purified by GLC on SE-30, OV-101, and Carbowax 20M columns. IR, NMR, and mass spectra were obtained on the purified pheromone. Additionally, it was hydrogenated and ozonized. All steps in the extraction, isolation, and purification were monitored by field bioassay. This simple bioassay consists of pouring 30 female equivalents (FE) of each fraction into a watch glass or petri dish placed on the ground in a peach orchard. The dishes are observed and the number of insects responding to each fraction is recorded.

Results: One pure compound that appears to have all the activity of the pheromone was isolated. About 30 FE (ca. 120 ng) of the natural pheromone has attracted as many as 50 males in 5 minutes. The pheromone was identified by spectroscopic and micro-degradative techniques. All the isomers are being synthesized.

Plans: All the synthesized isomers will be field tested this summer. Additionally, these compounds are being sent to Dr. David Nielsen (Ohio Agricultural Research Center, Wooster, Ohio) for field testing on related species of Aegeriidae. When the pheromone identity has been confirmed by synthesis, the synthetic pheromone will be tested by cooperating entomologists in trapping and control experiments to determine the best way to integrate it into the overall Peach Insect Management System.

<sup>1/</sup> C. E. Yonce and C. R. Gentry, Research Entomologists, Southeastern Fruit & Tree Nut Research Station Laboratory, P. O. Box 87, Byron, Georgia 31008





Isolation and Identification of the Sex Pheromone of the  
Khapra Beetle, Trogoderma granarium

K. Vick

Objective: To isolate and identify the sex pheromone of the khapra beetle using a novel bioassay technique.

Methods: Since the khapra beetle is not allowed in this country, a method of bioassay not based on khapra beetle males had to be developed. Since T. inclusum had previously been shown to be responsive to khapra beetle female sex pheromone extract, the decision was made to use T. inclusum males as the bioassay animals with the standard T. inclusum bioassay method. Female khapra beetles were collected over a two-year period in India and extracted with diethyl ether. This extract was then sent to this laboratory for purification and identification.

Results: The extract has been partially purified using gradient elution liquid adsorption chromatography. There are at least 3 chemicals in the extract that elicit sex pheromone response from T. inclusum males.

Plans: Each of the three chemicals that produce sex pheromone activity in T. inclusum males will be further purified by liquid and gas chromatography. Plans are being made to have some of the purified fractions bioassayed in India against khapra beetle males.



A Potent Sex Attractant for the Carpenterworm Moth,  
Prionoxystus robiniae

R. E. Doolittle, J. D. Solomon<sup>1/</sup>, W. L. Roelofs, and M. Beroza

Objectives: Isolate, identify and synthesize the female produced sex pheromone of the carpenterworm moth, Prionoxystus robiniae (Peck), the most destructive trunk boring insect in hardwood forests in the Southern United States. The pheromone may be useful in survey and control since the natural population of this insect is relatively low.

Methods: Abdominal tips of female insects and filter papers on which female insects had crawled were extracted, and the extracts purified by columnar, thin layer and gas liquid chromatography. Because rearing this insect is difficult, we used the electroantennogram screening method in the identification of the pheromone. Responses of males to the acetates of a series of mono-unsaturated fourteen carbon alcohols were recorded. Then the acetates of four diolefinic fourteen carbon alcohols were synthesized and field tested for their attractancy to males. During the 1973 emergence season, various new trap designs were tested.

Results: Preliminary field tests in 1972 indicated that of four possible isomers, only (Z-E)-3,5-tetradecadien-1-ol acetate was attractive. Attractiveness was potent and was enhanced by admixture of small amounts of (E-E)-3,5-tetradecadien-1-ol acetate as well as several mono-olefinic fourteen carbon alcohol acetates. In 1973, we confirmed these results. Since the synthetic route to the (Z-E)-isomer produces some of the (E-E)-isomer also, the reactive mixture may prove useful as an attractant without extensive purification.

Plans: Additional quantities of (Z-E)-3,5-tetradecadien-1-ol acetate will be synthesized for the 1974 emergence. Alternate more efficient synthetic routes to this compound will be explored. The pooled abdominal tips and filter paper extracts will be purified and fractionated in an effort to establish the identity of the natural pheromone as (Z-E)-3,5-tetradecadien-1-ol acetate.

<sup>1/</sup> J. D. Solomon, Forest Service Insect Research Laboratory, P. O. Box 227, Stoneville, Mississippi 38776



A Pheromone of the Japanese Beetle,  
Popilla japonica Newman,

J. H. Tumlinson, M. G. Klein<sup>1/</sup>, R. E. Doolittle, T. Ladd<sup>1/</sup>.  
J. DeVore, and R. R. Heath

Objectives: Isolation, identification, and synthesis of the pheromone produced by the female Japanese beetle, and its incorporation into an integrated control program for this pest.

Methods: Live female beetles are held in glass jars and fed apples. The beetles and apples are removed once a day and the jars rinsed with benzene. The vessel wash is concentrated by distillation and chromatographed on a Bio-Sil HA column eluted with pentane and ether. The active fractions collected from this column are further purified by GLC on OV-1, Carbowax 20M, OV-25, and Hi Eff 1 BP (DEGS) columns. NMR, IR, and mass spectra were obtained on the purified pheromone and it was ozonized. All steps in the isolation were monitored by field bioassay. Samples of material to be tested (usually 30 female days (FD) in about 0.3 ml solvent) are poured into glass petri dishes which are placed on the grass of open fields or golf courses.

Results: The pheromone has been isolated and tentatively identified on the basis of NMR, IR, and mass spectral data and micro-ozonolysis. The natural material consistently attracts large numbers of males in the field assays. In several instances, as many as 100 males have responded in 5-10 min to 30 FD of pheromone.

Plans: The pheromone will be identified and confirmed by synthesis and the natural and synthetic compounds will be compared for attractiveness in the field. Entomologists will then use the synthetic pheromone in trapping experiments and other investigations designed to develop a control program.

<sup>1/</sup> M. G. Klein and T. Ladd, Research Entomologists, Japanese Beetle Research Laboratory, Ohio Agricultural Research and Development Center, Wooster, Ohio 44691



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A Sex Pheromone of the Screwworm Fly,  
Callitroga hominivorax (Coquerel)

M. S. Mayer and D. A. Carlson<sup>1/</sup>

Objective: To attempt the identification of a sex pheromone produced by male screwworm flies.

Methods: The behavioral assays used in identification of the housefly sex pheromone were adapted for use with the screwworm fly.

Results: Preliminary results confirmed earlier observations that female flies are attracted to male flies. The response level was low, but statistical tests revealed highly significant responses occurred to fecal extracts and cuticle rinses over controls.

Plans: To begin full-scale assays for identification of the pheromone.

1/ Supported in part by transfer of funds from APHIS





## Sex Pheromone Studies of the Navel Orangeworm

J. A. Coffelt, K. W. Vick, and L. L. Sower

Objectives: To isolate and identify the female sex pheromone of the navel orangeworm, and ultimately, to evaluate the potential use of the sex pheromone of this species as a control or detection tool for wild populations infesting either wild or cultivated host materials. Therefore, we must develop an understanding of the physiological, behavioral and environmental factors which may influence the utility of the sex pheromone as a tool in the integrated control of this species.

Methods: A laboratory bioassay utilizing male activation as the response criterion was developed to monitor chemical isolation of the pheromone. The relationship between "attractancy" and "activation" was studied by comparing male response to different concentrations of crude pheromone in olfactometers designed to elicit the stimulation and/or upwind movement of the males. A series of extracts was prepared from unmated females of different ages, and of females of the same age at different times during a 24-hr cycle. Bioassay used the activation response criterion. Preliminary isolation procedures included solvent extraction of virgin female pheromone gland preparations, whole bodies of virgin females, or solvent extracts of filter papers upon which virgin females were held for differing lengths of time. Gradient elution liquid chromatography was the principal first step in the isolation procedure.

Results: A concentration of ca.  $1 \times 10^{-4}$  female equivalents (FE) was required to elicit a 50% response in test males with the male activation criterion. In contrast, in an olfactometer in which both activation and upwind movement could be observed, about 50% of the test males became active at  $1 \times 10^{-6}$  FE and approximately half oriented to the pheromone source at the concentration required for activation. Age-related changes in the pheromone content of 1-4-day-old females were noted. Two or 3-day-old females contained more extractable pheromone than did 1 or 4-day-old individuals. Among 1-2-day-old females, there were differences in pheromone content during a 24-hr cycle, peak recovery occurring at the time of night coincident with maximum female calling.

Plans: We will continue to process female moths for pheromone collection and examine additional techniques to improve the yield. We will study the role(s) of environmental, physiological and behavioral parameters in sex pheromone biology.



Isolation and Identification of the Sex Pheromone  
of the Angoumois Grain Moth

K. Vick, H. C. F. Su<sup>1/</sup>, L. L. Sower, P. D. Mahany<sup>1/</sup>,  
and P. C. Drummond

Objective: To isolate and identify the sex pheromone of the Angoumois grain moth.

Methods: The female sex pheromone was purified from female extract and its chemical nature characterized by spectrometric and chromatographic means. The sex pheromone was synthesized by Dr. Helen Su, Stored-Product Laboratory, Savannah, Georgia, and tested on male Angoumois grain moths.

Results: The male response elicited by the synthetic sex pheromone was identical to that elicited by purified natural sex pheromone both in laboratory bioassays and small scale field testing. The identity of the sex pheromone is thus confirmed as (Z-E)-7,11-hexadecadien-1-ol acetate.

Plans: This research has been completed.

Publication: Vick, K. W., H. C. F. Su, L. L. Sower, P. G. Mahany and P. C. Drummond. (Z-E)-7,11-hexadecadien-1-ol acetate: The sex pheromone of the Angoumois grain moth, Sitotroga cerealella. Submitted to Experientia

<sup>1/</sup> H. C. F. Su, Research Chemist, and P. G. Mahany, Physical Science Technician, Stored-Product Insects Research and Development Laboratory, ARS-USDA, P. O. Box 5125, Savannah, Georgia 31403.



Determination of the Evaporation Rates  
of Sex Pheromones

K. Vick, L. Sower, and D. Silhacek

Objectives: To systematically study evaporation rates of pheromones and pheromone-like substances from various substrates under different environmental conditions for support of experiments using pheromones for trapping and control.

Methods: The evaporation rates of  $C^{14}$ -labelled pheromones and pheromone-like chemicals are measured under different temperatures and air speed conditions and from different substrates. The alcohols of these chemicals are reacted with  $C^{14}$  acetic acid, and about 10-100 ng of this ester is applied to each substrate. At intervals after the application of the pheromone, the pheromone is extracted from the substrate and placed in a scintillation counter to determine the amount of radioactivity that was lost by evaporation. The effects of temperature, air velocity, molecular weight, pheromone quantity, and number of double bonds on evaporation are being studied.

Results: The project has recently been started, and only preliminary results have been obtained.

Plans: This study will be continued.



Pheromone Traps for the Angoumois Grain Moth

L. L. Sower and K. W. Vick

Objective: To develop an efficient, simple, and inexpensive survey trap for the Angoumois grain moth, Sitotroga cerealella (Olivier), using its sex pheromone as a bait.

Methods: Insects were introduced into a 20x20x8-ft. room as pupae in wheat kernels. Both sexes were present in approximately equal numbers. Sufficient amounts of infested wheat were continually added to maintain an adult population of about 150-250 insects. Sticky traps of different designs were placed in the room and baited with 1  $\mu$ g of synthetic sex pheromone applied to filter paper. Catches were monitored daily.

Results: The moths were most readily caught on horizontal sticky surfaces placed below the pheromone bait. Trap surfaces placed above or lateral to the pheromone caught considerably fewer insects. The efficiency of the traps is neither impaired nor enhanced if a roof is placed over the trapping surface containing the sex pheromone provided an open space of ca. 3 cm at the trap margin is maintained. Traps closed laterally, such as the Sector I or Howland type traps are much less efficient.

Plans: Investigations will be conducted to evaluate the effects of trap size and to test some additional trap configurations. A practical bait formulation will be found. The optimum sex pheromone release rate for the best trap will also be determined.





Cabbage Looper: Capture of Males in Traps Baited with  
Plastic Strips Impregnated with Pheromone

E. R. Mitchell, J. R. McLaughlin, M. Beroza<sup>1/</sup>, and D. L. Chambers

Objective: To evaluate different substrates for dispensing cabbage looper pheromone from traps.

Methods: Laminated plastic strips impregnated with synthetic pheromone, Z-7-dodecenyl acetate (prepared by Herculite Protective Fabrics Corporation, New York, N.Y.) were field tested in can and grid traps at Hastings, Fla., May 10-21, 1973. A polyethylene vial (1.25 ml) containing 0.1 ml pheromone was included as a control (Table 1).

Strips 2 X 3 cm of both thicknesses were the most effective for trapping cabbage looper moths with the can trap. In the can trap, there apparently was repellency of male moths due to a buildup of pheromone released from the thickest (1.0 mm) and largest (3 X 3 cm) laminate strip and from the vial. There were no significant differences among the same 7 treatments when used with the grid trap.

Plans: We will continue to evaluate various types of pheromone dispensers developed at this laboratory and/or supplied by commercial cooperators.

<sup>1/</sup> M. Beroza, Leader, Chemist, Organic Chemical Synthesis Laboratory, Beltsville, Maryland 20705



Table 1. Mean percentage of male cabbage looper moths captured in pheromone-baited can and electric grid traps.  
Hastings, Fla. May 10-21, 1973.

Description			Percentage of total moths captured	
			Trap design <sup>1/</sup>	
Thickness of laminate (mm)	Area of piece (cm)	Pheromone release rate (ng/min)	Can	Electric grid
<u>Herculite strips</u>				
0.5	1x3	16	14.6 a	10.5
	2x3	32	20.9 ab	14.9
	3x3	48	14.6 a	17.0
1.0	1x3	25	14.0 a	15.8
	2x3	50	26.6 b	16.4
	3x3	75	5.5 c	13.1
<u>Polyethylene vial<sup>2/</sup></u>				
		300	6.1 c	12.3

<sup>1/</sup> A total of 1797 and 5600 moths were captured with the can and grid traps, respectively. Means in the same column followed by the same letter do not differ significantly at the 5% level (Duncan's multiple range test). Absence of letters indicates nonsignificance between means.

<sup>2/</sup> Vial contained 0.1 ml pheromone.

Trap locations: In and around cabbage fields.

Replicates: Can, 2/treatment; grids, 1/treatment.

Treatment rotation: Captured insects were collected 2-3 times/week. Treatments were rotated 1 position after each collection.



Houseflies: Response to Muscalure in Poultry Houses

E. R. Mitchell, F. C. Tingle, and R. H. Guy

Objective: Determine the response of houseflies (Musca domestica) to muscalure-treated fiberboard blocks at different locations in and around poultry houses.

Methods: Fiberboard blocks (1 x 1 x 1/2 in.) treated with 1.5% muscalure in hexane were placed in aluminum pans (8 x 8 x 2 in.) containing ca. 1/2 oz Golden Malrin<sup>®</sup> fly bait and positioned on the ground adjacent to the manure and at 4 and 8 ft (ceiling height) above the ground along the outside edge or in the center of each of 3 poultry houses (total of 6 treatments/house). Flies were allowed to accumulate in the traps for 24 hr after which they were collected and counted. The test was repeated 3 times.

Results: Muscalure-baited traps were most effective when positioned at ground level adjacent to the manure (Table 1). Captures of flies decreased with increasing height above the ground. Only a few flies (<0.6% of total) were captured in traps located along the outside edge of the houses near the ceiling.

Plans: This phase of the research has been completed and no further work is contemplated.

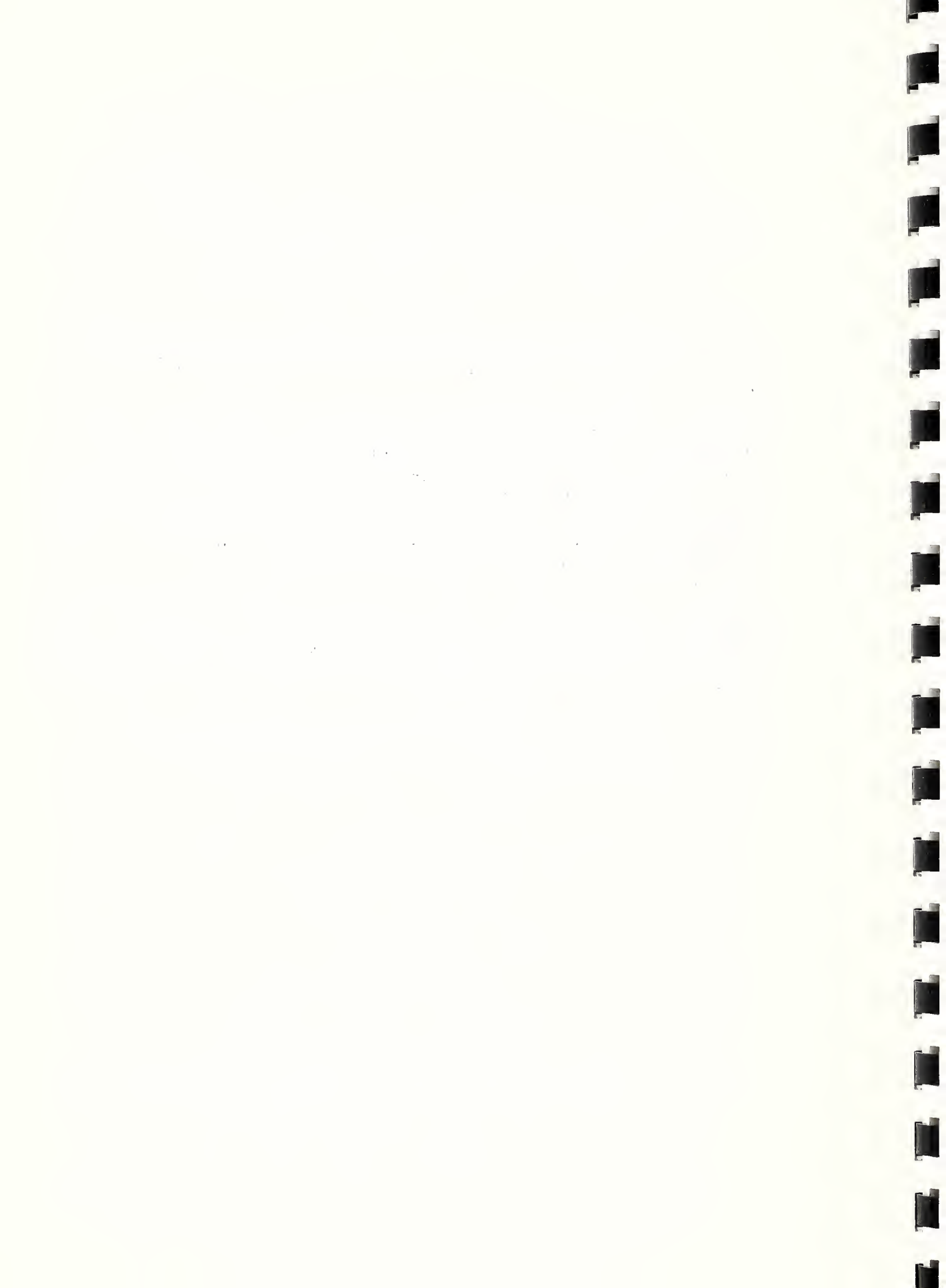


Table 1. Houseflies captured in poison-bait traps placed at different locations in poultry houses, Lake Butler, Fla., July 13-15, 1973.

Bait and trap location	Average no. flies/trap <sup>1/</sup>
<u>Outside edge</u>	
Sugar bait	
Ground	478 bc
4 feet	19 f
8 feet (ceiling)	6 f
Sugar bait and muscalure	
Ground	1271 a
4 feet	270 d
8 feet (ceiling)	23 f
<u>Center aisle</u>	
Sugar bait	
Ground	548 b
4 feet	85 ef
8 feet (ceiling)	70 ef
Sugar bait and muscalure	
Ground	1157 a
4 feet	341 cd
8 feet (ceiling)	220 de

<sup>1/</sup> Means followed by the same letter are not significantly different at the 5% level (Duncan's multiple range test).





## Control of Stored-Product Lepidoptera with Sex Pheromones

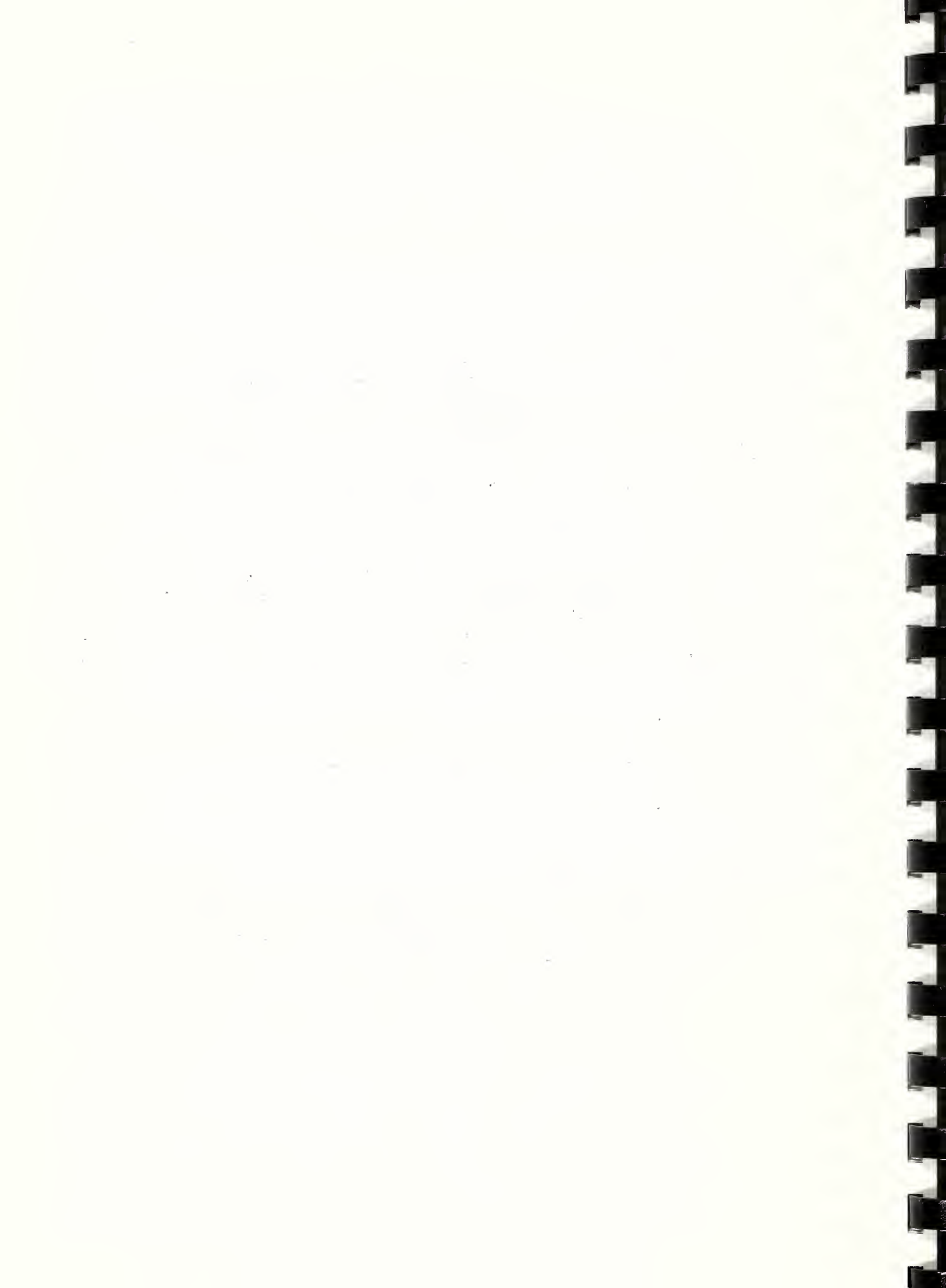
L. L. Sower, K. W. Vick and J. A. Coffelt

Objectives: To gather sufficient data to make a scientifically sound recommendation as to whether or not certain lepidopterous pests of stored products can be directly controlled by introducing synthetic sex pheromone into their environment. The immediate objective is to determine the effect of varying concentrations of synthetic sex pheromones on the mating frequencies of Indian meal moths and almond moths under controlled conditions consistent with a warehouse environment.

Methods: Each day 50 pairs of Indian meal moth pupae were introduced into a 20 x 20 x 8-ft room. The population of emerged adults was monitored daily for total numbers present, the male:female ratio, and the percent of females mated. These parameters became relatively stable at ca. 200 individuals; 45% were females, of which 65% had mated. Small quantities of sex pheromone were then introduced into the system. Each week the amount of pheromone was increased by about 10X based on the surface area of pheromone in contact with the air.

Results: Preliminary studies show a gradual reduction in the percent of females mated at any given time was correlated with increasing dosages of pheromone. About 20% of the females were mated in the presence of eight pheromone dispensers having a pheromone-treated surface area of about 15 sq. cm each.

Plans: The establishment of a relationship between pheromone dosage and percent of females mating will be completed. Then at least two additional variables will be considered: (1) The density of the insect population and (2) the distribution of pheromone dispensers. Similar experiments will then be repeated with the Angoumois grain moth and the almond moth using appropriate modifications.



Use of Sex Pheromones for Behavioral Control  
of Loopers and Related Noctuid Species

J. R. McLaughlin, E. R. Mitchell, and D. L. Chambers

Objectives: This study explores methods for controlling population levels by manipulating the sex pheromone communication systems of the cabbage looper, soybean looper, and several related species. The technique whereby the sex pheromone is continually evaporated into the air over a test area at a concentration above the male behavioral threshold (Environmental Permeation) is being examined. To adequately test this technique against wide-ranging species such as the cabbage and soybean loopers, large land areas must be treated. We are developing a system for broadcasting the pheromone over such areas.

Methods: We have concluded, based on preliminary field studies, that a broadcast method for dispensing the sex pheromone from many closely spaced sources is preferable to evaporating the chemical from fixed stations located at predetermined intervals. We are now formulating the pheromone on substrates including string, wood chips, dust, fibre blocks, plastic polymers, mineral and vegetable oils, and clay-based polymeric gels. Included in the tests are compounds which may reduce the rate of oxidative degradation of the pheromone. These substrates are being broadcast in field plots, used as trap baits, or examined in laboratory assays (behavioral, GLC, radiometric) to determine their effectiveness in disrupting sex pheromone communication, their evaporation and degradation characteristics, toxicity to crops and farm lands, handling characteristics, and potential cost.

Results: Wood chips (pine, ca. 5 x 15 x 50 mm, treated with 0.1 ml of pheromone) and string (24 ply x 50 mm, treated with 0.1 ml of pheromone) were attractive to male cabbage loopers for up to 73 days in the field when the pheromone was protected by small amounts of antioxidants. Preliminary laboratory evaluation of clay-based gels indicates that this formulation should undergo extensive field testing.

Plans: This is a high priority project. Field populations of the appropriate insects are just becoming available and extensive field testing should be possible from now until next June. Much of our effort will be directed toward adequate measurement of the impact of our treatments on looper populations.



Modification of Sex Pheromone Responses  
by Pheromone-like Compounds

L. L. Sower, K. W. Vick, and J. H. Tumlinson

Objectives: Certain compounds are known to modify the sex pheromone responses of some moths. We wish to determine the effects and significance of such compounds relative to the biology of certain lepidopterous pests of stored products.

Methods: Bioassay procedures and chemical isolation and identification methods common to this laboratory were used.

Results: (Z-Z)-9,12-tetradecadien-1-ol acetate was found to inhibit the sex pheromone responses of the male Indian meal moth, Plodia interpunctella (Hübner). Evidence was found which strongly supports the contention that (Z-E)-9,12-tetradecadien-1-ol acetate is not the only stimulatory sex pheromone released by female Indian meal moths. (Z-E)-9,12-tetradecadien-1-ol was isolated and identified from female Indian meal moths; this compound inhibits the sex pheromone responses of male almond moths, Cadra cautella (Walker). Bioassay tests indicate that the insects can habituate to the inhibitory compounds without losing their ability to respond to the sex pheromones.

Plans: This project is currently inactive but will be reactivated when certain other projects are completed.

Publications: Vick, K. W. and L. L. Sower. Z-9,Z-12-tetradecadien-1-ol acetate, an inhibitor of the sex pheromone response of Plodia interpunctella (Hübner). (Submitted to J. of Econ. Ent.)

Sower, L. L., K. W. Vick, and K. A. Ball. Perception of olfactory stimuli that inhibit the responses of male moths to sex pheromones. (National Technical Editor)

Sower, L. L., K. W. Vick, and J. H. Tumlinson. (Z,E)-9,12-tetradecadien-1-ol: A chemical released by female Plodia interpunctella (Hübner) that inhibits the pheromone responses of male Cadra cautella (Walker). (National Technical Editor)



Evaluation of Inhibitor for Behavioral Control  
of Cabbage Loopers and Soybean Loopers

J. R. McLaughlin and E. R. Mitchell

Objectives: To evaluate Z-7-dodecen-1-ol which inhibits the response of male cabbage loopers to the sex pheromone, Z-7-dodecen-1-ol acetate (looplure), in field traps and laboratory flight tunnels, as an agent to control mating in field populations of the cabbage looper and soybean looper using the environmental permeation technique.

Methods: We dispensed the chemicals from polyethylene vials with an evaporation rate (27°C, 0.4 m/sec windspeed) of ca. 300 ng/min per dispenser. Field plots consisted of 4 x 4 checkerboard grid arrangements of dispensers hung 1m from the ground at 30X30-m intervals. We placed a can trap baited with one dispenser of looplure or with 10 virgin female cabbage loopers at the center of each grid. Treatments consisted of a grid of inhibitor dispensers, a grid of looplure dispensers, a grid with both inhibitor and looplure dispensers in an alternating arrangement, and a control trap not surrounded by the chemicals. The plots were in fields of lupine near the campus of the University of Florida, Gainesville, and later in cabbage fields near Hastings, Fla. The traps were inspected daily (except weekends) and treatments were rotated from one trap location to the next every Monday and Thursday. The test began with each trap baited with females. For the second night, each trap bait was a looplure dispenser, and this alternation of baits was maintained throughout the test.

Results: The results are summarized in Tables 1 and 2. We conclude that the inhibitor does not interfere with the ability of male cabbage loopers or soybean loopers to locate a point source of looplure (or pheromone-releasing females) when the inhibitor is evaporated into the atmosphere surrounding the source of attraction.

Plans: Inhibitor no longer considered promising for behavioral control. Will continue study on reduced level in relation to the soybean looper and will conduct further behavioral studies with cabbage looper if the enzyme studies of Mayer and Ferkovich seem to warrant such an effort.







Table 1. Mean number ( $\pm$ SE) of cabbage looper males captured each night in traps baited with looplure or virgin cabbage looper females and placed within grids of evaporators of looplure (P) or inhibitor (I).

Grid Treatment	No. captured in traps baited with: Virgin Females (n=12 nights)	Looplure (n=11 nights)
P	0.0	0.9 $\pm$ 0.6
PI	0.2 $\pm$ 0.1	0.6 $\pm$ 0.3
I	3.3 $\pm$ 2.0	5.2 $\pm$ 2.9
None	1.4 $\pm$ 0.9	4.7 $\pm$ 2.5

Table 2. Mean number ( $\pm$ SE) of soybean looper males captured each night in traps baited with looplure and placed within grids of evaporators of looplure (P) or inhibitor (I).

Grid Treatment	No. males captured each night (n=11 nights)
P	0.3 $\pm$ 0.1
PI	0.5 $\pm$ 0.3
I	4.5 $\pm$ 2.2
None	4.2 $\pm$ 2.8



Cabbage Looper: Interaction of Blacklight,  
Sex Pheromone, and Pheromone Inhibitor

E. R. Mitchell, D. L. Chambers, and J. H. Tumlinson

Objective: The capture of male Trichoplusia ni in traps baited with virgin female cabbage loopers or their synthesized pheromone, Z-7-dodecen-1-ol acetate (looplure), is greatly reduced when Z-7-dodecen-1-ol (inhibitor) is dispensed simultaneously from the same trap. The mechanism of this inhibition of pheromone perception by males is unknown though it is strongly suspected that the pheromone and inhibitor are perceived at different sites. The effect, if any, of inhibitor on wild female cabbage loopers is unknown. A chemical that repels moths would be useful for control especially if the repellent would prevent females from entering, mating, and/or ovipositing in a treated area. We, therefore, conducted a field experiment at Hastings, Fla., during spring 1973 to determine if the inhibitor exhibits any repellency toward cabbage loopers attracted to blacklight. Blacklight (BL) was used as bait because it attracts both sexes of the cabbage looper moth.

Methods: Eight 15-watt BL traps conforming to ESA standards were baited as follows (2 replicates/treatment): (a) 0.1 ml looplure; (b) 0.1 ml inhibitor; (c) the two combined; and (d) a BL control. The chemicals were dispensed from 1.25 ml polyethylene vials midway and ca. 3 cm from the BL source. Separate vials were used for dispensing looplure and inhibitor in the combined treatment. The captured insects were collected, sexed, and counted every 2-3 days during the test period (Apr. 30-May 11). The vials were rotated 1 position after each collection among permanent trapping stations (0.5-1 mile apart) located in and around cabbage fields.

Results: The inhibitor had no apparent effect on the capture of male or female cabbage looper moths with blacklight traps (Table 1). These results indicate that the inhibitor is very specific in action apparently effecting only pheromone perception by males.

Plans: The inhibitor will be tested in similar fashion against soybean loopers.



Table 1. Captures of cabbage looper moths in blacklight traps baited with Z-7-dodecen-1-ol acetate (looplure) or Z-7-dodecen-1-ol (inhibitor). Hastings, Fla., Apr. 30-May 11, 1973.

Bait	<u>Mean % of Total Moths Captured</u> <sup>1/</sup>	
	Male	Female
0.1 ml looplure	55.3a	27.5
0.1 ml inhibitor	14.4b	22.2
0.1 ml looplure + 0.1 ml inhibitor	15.1b	23.8
BL Alone	15.2b	26.8

<sup>1/</sup> 765 males and 785 females were captured. Means followed by the same letter do not differ significantly at the 5% level (Duncan's multiple range test). The absence of letters indicates nonsignificance between means.



Effect of Inhibitor on the Response of  
Male Cabbage Loopers to Sex Pheromone

J. R. McLaughlin, M. S. Mayer, and E. R. Mitchell

Objective: To study the behavioral effect of Z-7-dodecenol, which inhibits the response of male cabbage loopers to the sex pheromone, Z-7-dodecen-1-ol acetate (looplure), when the chemicals are evaporated from the same locus although we found no reduction in the response of males to looplure or virgin females in an inhibitor-permeated field environment.

Methods: In the field, can traps were baited as follows with vials releasing the chemical at ca. 300 ng/min: 1 of looplure and 1 of inhibitor at the usual bait position; 1 of inhibitor wired to the screened top of the trap and 1 of looplure in the usual position; and 1 of looplure in the usual position. Laboratory bioassays were conducted in plexiglass wind tunnels at .25 m/sec windspeed, 27°C, 50% RH, and 0.5 lux light level. Looplure or inhibitor were released from one or more of 3 points upwind of the holding compartment in each tunnel: (A) 277 cm, (B) 268 cm, and (C) 138 cm. The chemicals were coated (0.1 µg) on the inside of glass tubes and evaporated into the tunnels in 50 ml/min of air. Three-day-old males were released from the holding compartment for 30 sec after the chemical laden air reached them and then the tunnels were divided into 3 areas: (1) 27 cm-long (original holding compartment), (2) 238 cm-long (including point C), and (3) the remaining upwind 85 cm of each tunnel (including points A&B). Twenty males were released in each of 5 replicates for each test. In one series of tests the males were held in a closed container and exposed to large doses of looplure or inhibitor for 30 min, 1 or 2 hr and then flown in the tunnels within 5 min of their removal from this treatment.

Results: The inhibitor markedly reduced the capture of males in the field (Table 1) when evaporated at the same locus as looplure in the traps, but was less effective when removed from that locus by only ca. 30 cm. The conclusion that the inhibitor is not a repellent and does not inhibit the upwind flight response of males to looplure is confirmed by the behavior of males in the wind tunnels (Table 2). Males readily flew upwind toward looplure in the presence of inhibitor (L,M,N,O). When inhibitor was near or at the same locus as looplure (L,N,O) the males failed to orient to and to be held at the point source of the pheromone. The inhibitor does not habituate the response to looplure (F,H,K) which suggests very strongly that the inhibitor and looplure are perceived at different receptor sites. Preliminary electroantennogram data support the dual receptor supposition which is parallel to that developed for Plodia and Cadra by Dr. Sower.





Plans: Wind tunnel studies will be expanded to determine the effects of windspeed, temperature, humidity, visual patterns, light levels, and preexposure to pheromone on the response of cabbage looper and soybean looper males to Z-7-dodecen-1-ol acetate and female extracts.

Table 1. Mean ( $\pm$ SE) of male cabbage loopers captured per trapping interval (1-3 nights) in traps baited with looplure and inhibitor.

Position of Inhibitor	Mean Males/Interval (n=63 intervals)
Same locus as looplure	0.08 $\pm$ 0.03
Top of trap	1.8 $\pm$ 0.5
None	4.5 $\pm$ 0.9

Table 2. Response of male cabbage loopers to looplure (P) and inhibitor (I) in flight tunnels.

Test	Chemical at Point:			Preexposed for:(hrs)			Mean ( $\pm$ SE) in compartment:		
	A	B	C	0.5	1.0	2.0	1	2	3(Upwind)
D	P	-	-	-	-	-	8 $\pm$ 5	25 $\pm$ 6	67 $\pm$ 7
E	P	-	-	P	-	-	61 $\pm$ 5	19 $\pm$ 4	20 $\pm$ 5
F	P	-	-	I	-	-	18 $\pm$ 7	14 $\pm$ 4	68 $\pm$ 6
G	P	-	-	-	P	-	67 $\pm$ 10	16 $\pm$ 6	17 $\pm$ 9
H	P	-	-	-	I	-	15 $\pm$ 5	14 $\pm$ 5	71 $\pm$ 5
J	P	-	-	-	-	P	72 $\pm$ 7	23 $\pm$ 6	6 $\pm$ 5
K	P	-	-	-	-	I	15 $\pm$ 4	21 $\pm$ 6	61 $\pm$ 8
L	PI	-	-	-	-	-	13 $\pm$ 4	50 $\pm$ 10	38 $\pm$ 11
M	P	-	I	-	-	-	7 $\pm$ 4	24 $\pm$ 3	69 $\pm$ 4
N	P	I	-	-	-	-	14 $\pm$ 4	24 $\pm$ 3	49 $\pm$ 1
O	I	P	-	-	-	I	22 $\pm$ 9	38 $\pm$ 6	47 $\pm$ 7
Q	PI	-	-	-	-	-	10 $\pm$ 3	30 $\pm$ 6	61 $\pm$ 8



Fall Armyworm: Inhibition of Pheromone Perception  
by Males with Synthetic Acetates

E. R. Mitchell, W. W. Copeland, A. N. Sparks<sup>1/</sup>, A. A. Sekul<sup>1/</sup>

Objective: To evaluate the effects of different chemicals on attraction of fall armyworm males to females and synthetic pheromone, based on the observation that cabbage looper pheromone inhibits this attraction.

Methods and Results: Experiment 1 -- Saucer-type traps constructed from 10-in. styrofoam pie plates and coated with Stickem were baited with 3 virgin female fall armyworm moths (2 days old) and 30 mg each of dodecyl acetate (da), Z-7-dodecen-1-ol (Z-7-dd), Z-7-dodecen-1-ol acetate (Z-7-dda), or Z-9,trans-12-tetradecadien-1-ol acetate (Z-9,trans-12-tda). The chemicals were dispensed from cotton wicks attached to the females' cages; each was replicated 3 times, and positioned ca. 30 m apart in randomized complete blocks along the edge of a sorghum field near the Attractants Laboratory. Captured insects were collected and counted daily after which the treatments were rotated 1 position.

Dodecyl acetate and Z-7-dd had no effect on the attraction of male fall armyworm moths to virgin females. However, both Z-7-dda and Z-9,trans-12-tda reduced the attraction of males to females 90-100% (Table 1).

Experiment 2 -- The chemicals used in the 1st experiment were at least 95% pure. Z-7-dda and Z-9,trans-12-tda therefore were diluted with da (not biologically active) to give solutions of 1, 10, 25, and 50% by volume which were tested to determine concentration effect on the attraction of males to Z-9-dodecen-1-ol acetate (Z-9-dda), the synthetic sex pheromone of the fall armyworm. The chemicals (50 mg each) were dispensed from separate 1.25 polyethylene vials attached to saucer traps as described above. The treatments were spaced ca. 30 m apart in randomized complete blocks (3) along the edge of a cornfield near the laboratory and observed and rotated as described. As little as 10% Z-7-dda or Z-9,trans-12-tda in da reduced captures of male fall armyworm moths in pheromone baited traps 93-95% (Table 2).

Plans: The results suggest that Z-7-dda and Z-9,trans-12-tda may be useful for disruption of pheromone communication in the fall armyworm. Small plots will be treated with these and other synthetic chemicals to test this potential.

<sup>1/</sup> A. N. Sparks, Res. Entomol. and A. A. Sekul, Res. Chemist,  
Southern Grain Insects Research Laboratory, Tifton, Georgia 31794



Table 1. Effects of different chemicals on the attraction of fall armyworm (FA) males to virgin females. Gainesville, Fla., 1972

Bait <sup>a/</sup>	Mean no. males captured/trap <sup>b/</sup>			
	9/2	9/3	9/4	9/5
3 FA females	29.7 a	18.7 a	7.0 a	6.7 a
3 FA females +				
30 mg da	27.3 a	14.3 a	4.3 a	3.8 a
30 mg <u>Z</u> -7-dd	19.3 a	16.7 a	6.3 a	3.3 a
30 mg <u>Z</u> -9, <u>trans</u> -12-tda	0.7 b	3.3 b	.7 b	.7 b
30 mg <u>Z</u> -7-dda	0 c	0 c	0 c	0 c

<sup>a/</sup> da = dodecyl acetate; Z-7-dd = Z-7-dodecen-1-ol; Z-9,trans-12-tda = Z-9,trans-12-tetradecadien-1-ol acetate; Z-7-dda = Z-7-dodecen-1-ol acetate.

<sup>b/</sup> Three replications/treatment per night. Means in the same column with letters in common are homogenous (P=0.05, Duncan's multiple range test).



Influence of Wavelength and Intensity of Light on  
Flight and Mating Activity of Cabbage Looper Moths

W. K. Turner and N. C. Leppla

Objective: To study the influence of color and nocturnal intensity of light on the amount of flight activity and reproductive success of the cabbage looper, Trichoplusia ni.

Methods: Populations of 50 male and 50 female cabbage looper moths 1-2 days old were held 2 days under various colors and nocturnal intensities of light. CO<sub>2</sub> produced by the moths was measured and served to indicate activity. Data on reproduction included percent of females mated and spermatophores per mated female. Data shown under results are from the final 24 hours of the tests. Sixteen treatments were applied, consisting of four nocturnal intensities for each of four colors, with a single combination of color and nocturnal intensity used in each test. Light colors were gold, blue, uv (BLB) and white (Vitalite®). Diurnal intensities (watts/cm<sup>2</sup>) for all colors were made equal with neutral density filtering. Two 15-watt fluorescent lamps were used for each test. Gold lamps had the least energy output, and served as the standard. Nocturnal levels were obtained with a cam operated shutter and were 0, 1, 10 or 100% of the diurnal intensity, with gradual transitions to stimulate natural conditions. Additional tests with continuous darkness were conducted. Nocturnal and diurnal temperatures were 75 and 80°F, respectively; transitions again were gradual.

Results: The mating data (Figs. 1 & 2) show high mating success under continuous dark and continuous UV. The white light appeared to inhibit mating more than the other colors. Nocturnal flight activity (Fig. 3) under UV remained high even when the nocturnal UV was 100% of the diurnal level. Diurnal activity (Fig. 4) was greatest under UV, but also high under continuous darkness. Trends are indicated, but more study is needed to confirm all but the greatest differences.

Plans: Work will continue toward defining the behavior differences under UV as compared with the visible radiations. Egg hatch is being added as a behavior indicator.





MATING OF CABBAGE LOOPER MOTHS  
MAINTAINED UNDER DIFFERENT COLORS OF  
LIGHT AND DIFFERENT INTENSITIES OF  
NOCTURNAL LIGHT

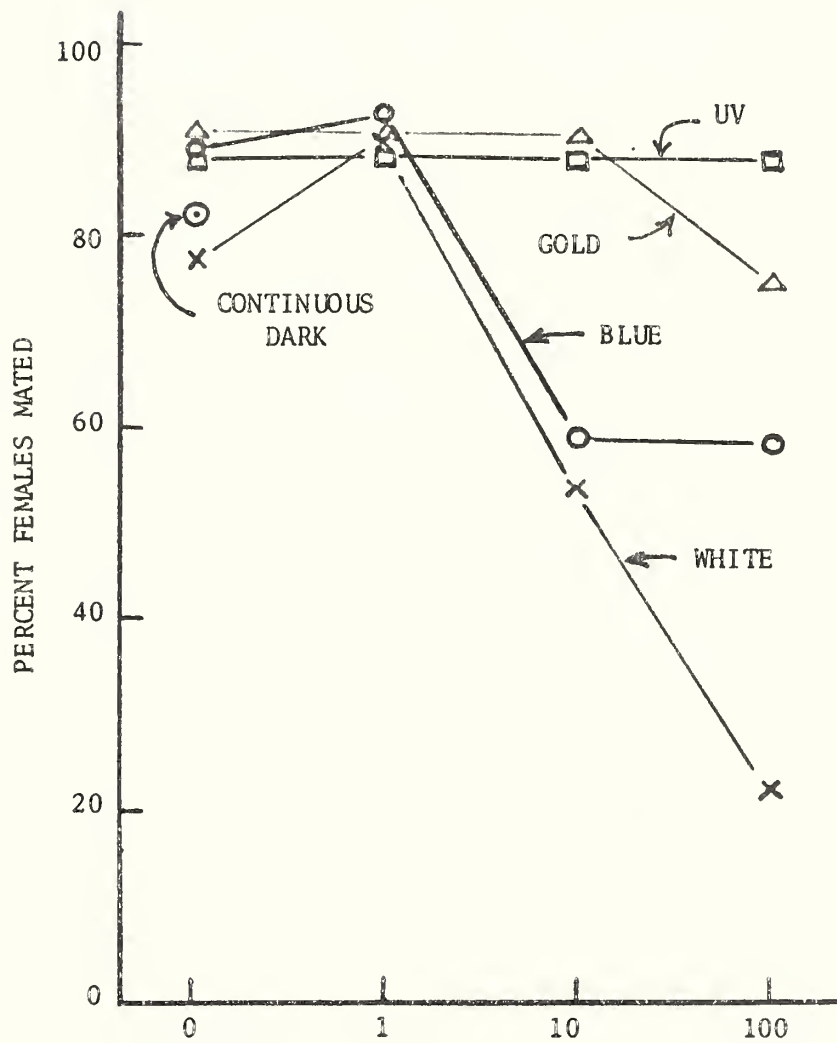


FIG. 1. NOCTURNAL LIGHT INTENSITY (% OF DIURNAL INTENSITY)



MATING OF CABBAGE LOOPER MOTHS MAINTAINED  
UNDER DIFFERENT COLORS OF LIGHT AND  
DIFFERENT INTENSITIES OF NOCTURNAL LIGHT

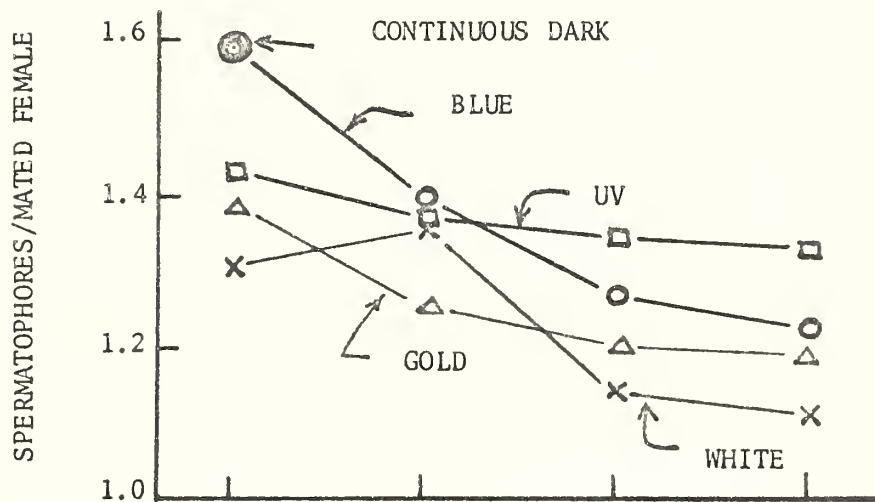


FIG. 2. NOCTURNAL LIGHT INTENSITY (% OF DIURNAL INTENSITY)



NOCTURNAL ACTIVITY OF CABBAGE LOOPER  
MOTHS MAINTAINED UNDER DIFFERENT COLORS OF  
LIGHT AND DIFFERENT INTENSITIES OF NOCTURNAL LIGHT

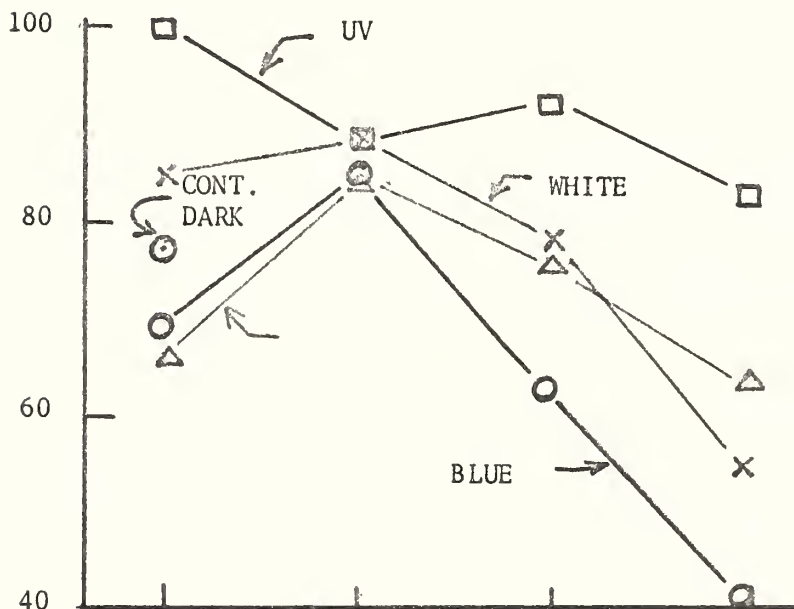


FIG. 3. NOCTURNAL LIGHT INTENSITY (% OF DIURNAL INTENSITY)

DIURNAL ACTIVITY OF CABBAGE LOOPER  
MOTHS MAINTAINED UNDER DIFFERENT  
COLORS OF LIGHT

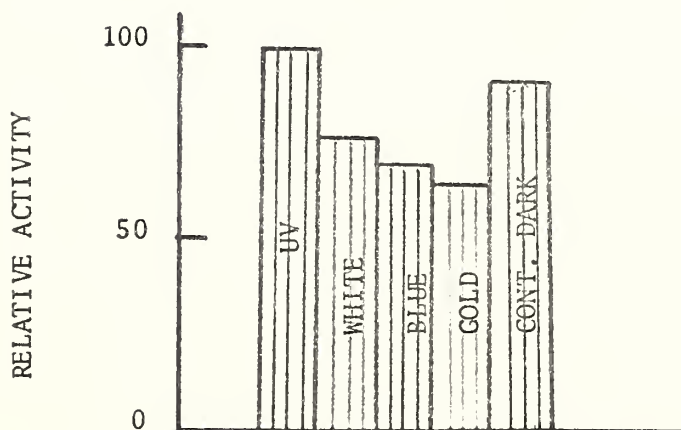


FIG. 4. COLOR



Response of Cabbage Looper and Corn Earworm Moths  
to Far Infrared Laser Radiations

W. K. Turner, P. S. Callahan and F. Lee

Objective: To determine whether or not cabbage looper (Trichoplusia ni) and corn earworm (Heliothis zea) adults sense and respond to far infrared (IR) laser radiations, with wavelengths of 28, 118, and 337  $\mu\text{m}$ . Although preliminary tests at this laboratory did not show responses, it has been reported that corn earworm and fall armyworm adults respond to wavelengths of 118 and 337  $\mu\text{m}$  (Eldumati and Levengood, 1971, Nature 233:283).

Methods: Laboratory reared cabbage looper and corn earworm adults were irradiated with IR laser radiation of 28, 118 and 337  $\mu\text{m}$  wavelengths. Hydrogen cyanide and water vapor gas lasers were used. Two methods were used to monitor responses. For one, groups of insects were placed in a long plexiglass tube, and the IR radiation directed along the longitudinal axis of the tube. Distribution within the tube was recorded periodically. The other method used the  $\text{CO}_2$  production of the test insects as an indicator of response.  $\text{CO}_2$  was monitored continuously and the IR radiation was presented periodically.

Results: There was no apparent response of corn earworm or cabbage looper adults to the far IR laser wavelengths tested. The "normal" respiratory activity of male and female cabbage loopers was not altered by periodic irradiation with 118 and 337  $\mu\text{m}$  IR. Also, there was no evidence that the three far IR frequencies influenced the distribution of either insect species within the plexiglass tube.

Plans: More tests will be conducted with cabbage looper and corn earworm at the three IR frequencies named above. The study will be broadened to include other insect species and additional wavelengths around 5 and 10.6  $\mu\text{m}$ . Provided responses are found; efforts will be made to relate this sensing capability to the insect's communication and host location abilities.





Effect of Various Colors on the Response of Male Cabbage  
Loopers to Double-cone Type Can Traps

J. R. McLaughlin and E. R. Mitchell

Objective: Because of the availability of prepainted lard cans, Shorey and coworkers have always used green double-cone type can traps baited with cis-7-dodecenyl acetate and other sex pheromones and parapheromones to trap noctuids in California. Our local supply of cans for these traps are not painted and rust quickly if not protected. As we must paint these cans ourselves, we explored the effect of several colors on our catches.

Methods: The modified double-cone traps (Kaae and Shorey, 1972, Environ. Entomol. 1:675-77) were constructed from 50 lb lard cans (Glover Wholesale Co., Americus, Ga.) and painted black, fluorescent red, fluorescent yellow, fluorescent orange, and silver. Three trap types were painted green; one had the clear plexiglass top recommended by Kaae and Shorey, one had a wire screen top, and the other a solid metal top. These traps were baited with a dispenser releasing ca. 300 ng/min of cis-7-dodecenyl acetate and were placed at 50-m intervals in rows in cabbage fields, checked every 2nd or 3rd day, and rotated in order.

Results: Table 1 summarizes the data. Green appears to be one of the least desirable colors. The high degree of human visibility would make the fluorescent orange trap most desirable for field use. Silver traps would presumably reflect heat and better protect females used as bait; however, most silver (aluminum) paints tend to stain clothing and hands rather easily. Wire-topped traps are more cheaply and easily constructed and maintained than plexiglass or acetate-topped traps. It appears that light must be allowed to pass through the top of the trap.

A side-by-side comparison of the can-type traps previously used at the Gainesville lab against the recent modification of Kaae and Shorey showed the new design to be far superior in trapping cabbage looper (Trichoplusia ni (Hübner)) and soybean looper (Pseudoplusia includens (Walker)) males.

Plans: The development of improved pheromone traps for noctuids is an ongoing effort. Studies this fall and winter of the behavior of pheromone-releasing females in the field and the response of males to these females may suggest other trap designs or aid in the proper deployment of pheromone traps.



Table 1.--Effect of color and top design on catches of male cabbage loopers in double-cone (can) traps releasing loop lure at ca. 300 ng/min.

Trap design	Mean ( <u>+SE</u> ) of males trapped per interval (2-3 nights), n=19 intervals
Black	10 <u>+4</u>
Red	9 <u>+4</u>
Yellow	10 <u>+6</u>
Orange	16 <u>+8</u>
Silver	20 <u>+10</u>
Green (plexiglass top)	7 <u>+5</u>
Green (wire top)	10 <u>+3</u>
Green (solid top)	0



# Catch of Houseflies Influenced by Color of Sex-Lure Traps

F. C. Tingle, E. R. Mitchell, A. Q. Antonio, R. H. Guy and D. Carlson

**Objectives:** To determine the attractance of houseflies to selected colors under the existing conditions of a poultry farm, and the interaction of color and attractance by the housefly sex attractant, muscalure.

**Methods:** Sticky Formica<sup>®</sup> panels (10" x 12") painted one of 12 selected colors (Table 1) were exposed at a poultry farm to determine the color preference of houseflies. They were placed randomly 8' apart from 1600-1900 in 4 tests conducted in September 1972. Attraction to color alone was measured in tests 1 and 2.

In the 3rd test, muscalure was tested in combination with the same colors used in tests 1 and 2. Painted aluminum pie pans were used instead of the panels, and a bait consisting of Dipterex<sup>®</sup> and DDVP combined with sugar and moistened with water was brushed onto the pans. Muscalure on corn cob grits at the rate of 5 mg/trap was spread over the surface of each pan.

The 4th test consisted of the 2 best treatments (white and aluminum foil) from tests 1 and 2 and the 3 best treatments (black-flat, tangerine, and orange) from the 3rd test. Each color was tested with bait with and without muscalure. All treatments in all tests were replicated 3 times.

**Results:** White and aluminum foil covered panels attracted significantly more houseflies in identical tests conducted on successive weeks, than did the other colored panels (Table 1).

When muscalure was used in combination with color, there was no significant difference in the number of flies trapped in the different colored traps (Tables 1 and 2) and the addition of muscalure increased fly capture.

No significant sex preferences for color were observed, and the presence of muscalure in the traps had little effect on the sex ratio of flies captured.

**Plans:** Information from these tests will be used in further testing of muscalure for various methods of housefly control.



Table 1.--Comparison of the efficiency of colored traps in capturing houseflies. Starke, Fla. 1972.

Trap color	Average number flies/trap <sup>a/</sup>		
	Tests 1 and 2 (Sticky panels) <sup>b/</sup>		Test 3
	8/31/72	9/6/72	(Pie pans) 9/11/72
White	610 a	55 a	381
Aluminum foil	576 a	59 a	432
Navy gray	420 ab	18 bc	309
Blue	294 bc	17 bc	418
Black (Hi-Gloss)	194 c	15 c	413
Solar Yellow	184 c	48 ab	369
Black (Flat)	169 c	22 bc	489
Tangerine	168 c	15 c	487
Chesnut Brown	160 c	12 c	372
Vibrant Green	137 c	9 c	420
Coment Orange	95 c	29 abc	468
Brilliant Cerise	91 c	16 bc	418

<sup>a/</sup> Muscalure was not included in tests 1 and 2. Traps in test 3 were baited with 5 mg muscalure each.

<sup>b/</sup> Means in the same column followed by the same letter do not differ significantly at the 5% level (Duncan's multiple range test). The absence of letters indicates nonsignificance between means.





Table 2.—The effect of muscalure on the capture of houseflies. Starke, Fla. 1972.

Trap Color	Average number flies/trap <sup>a/</sup>	
	With 5 mg muscalure/trap	Without muscalure
Aluminum foil	209 a	54 b
Black (Flat)	201 a	74 b
Unpainted White	162 a	55 b
Tangerine	157 a	36 b
Comet Orange	152 a	45 b

<sup>a/</sup> Means followed by the same letter do not differ significantly at the 5% level (Duncan's multiple range test).



Analysis and Identification of Specialized Signalling  
Sound Produced by the Male Caribbean Fruit Fly

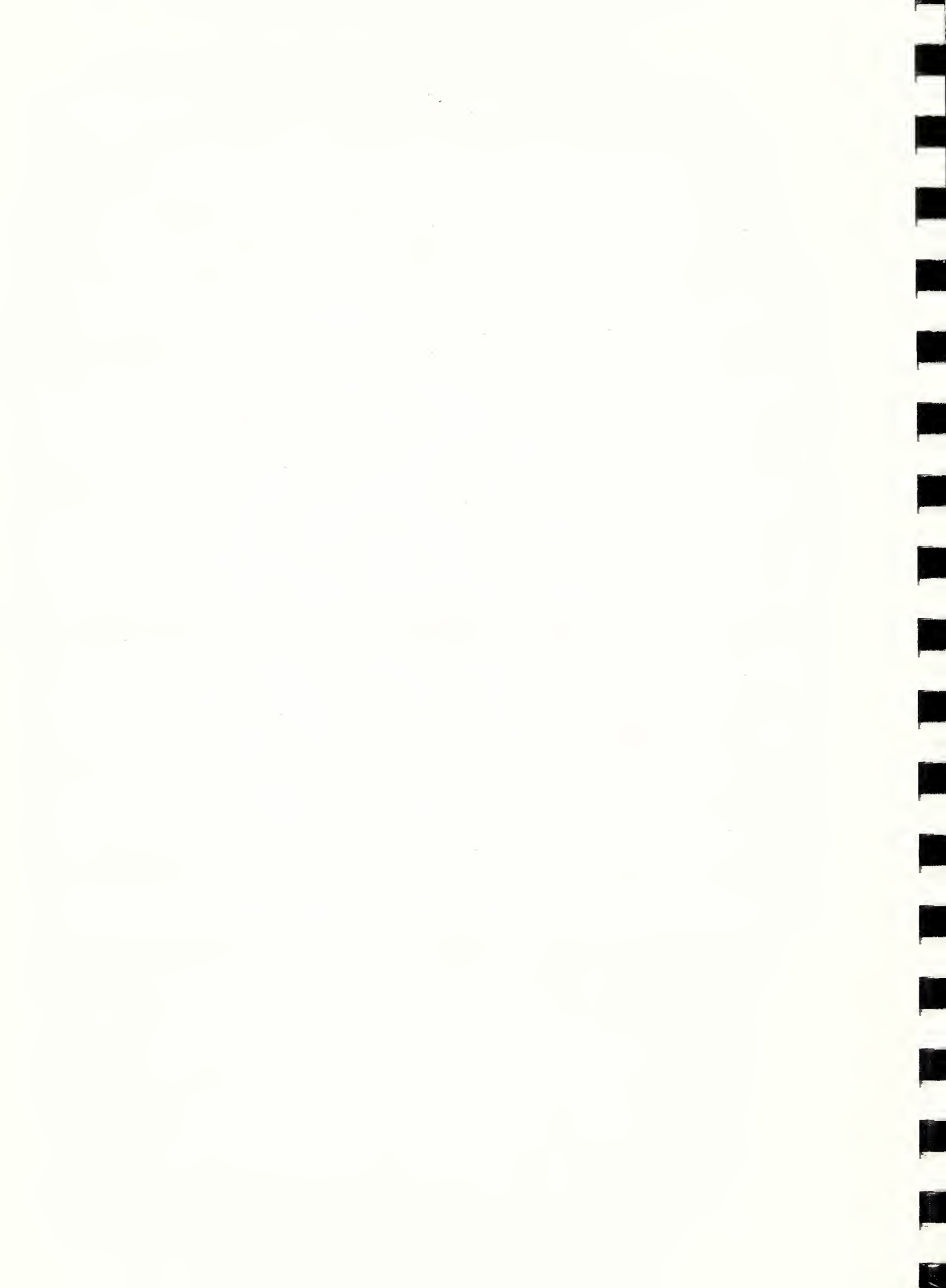
J. C. Webb, J. L. Sharp, D. L. Chambers, and J. C. Benner

Objectives: To isolate and identify the specialized "signalling" sound produced by the male Caribbean fruit fly (Anastrepha suspensa) before pair formation.

Methods: The sounds that are produced by the fruit fly incidental to ordinary movement were isolated and identified. These sounds included flight, aggression, walking, preening, etc. Also, the specialized sound produced by the signalling male was recorded. All miscellaneous sounds were then removed from the calling sound which allowed a more accurate analysis and identification of the information contained within the signalling sound. A bioassay test unit was constructed to measure the response to these sounds of both males and females. Response comparisons made in the test were: males to males; males to reproduced sound; males to pheromone; males to pheromone plus reproduced sound; females to calling males; females to pheromone; females to reproduced sound; and females to pheromone plus reproduced sound.

Results: The sounds resulting from incidental movements contained both low and high frequency components. The low frequency components were mainly wingbeat sounds and ranged from 100 to 200 Hz. The high frequency sounds were in the form of pulses and the frequency ranged from 20 to 50 kHz. The calling sound consists of low frequency pulses. The physical characteristics of the signalling sound are in the process of being analyzed. Preliminary data show that the following treatments are not different from each other but are significantly different from the other 5 treatments: females to extracted pheromone; females to males; and females to reproduced signalling sounds.

Plans: To determine if this signalling sound is an important behavioral cue and useful in manipulating the fly in the laboratory or field.



Acoustical Properties of the Flight Activities of the  
Caribbean Fruit Fly

J. C. Webb, J. L. Sharp, D. L. Chambers, and J. Benner

Objectives: To determine the acoustical properties of the flight activities of the Caribbean fruit fly, Anastrepha suspensa (Loew) and relate these properties to fly sex and age.

Methods: The flies were attached singly to a rotor arm of a flight mill which was located in an anechoic chamber. A very sensitive microphone located 1/2 in. below the fly detected the wingbeat sounds generated by the flies in a stationary position and in circular flight. These sounds were then recorded and analyzed for frequency content, harmonic properties, sound pressure level, and flight velocity. These acoustic characteristics were correlated for both sexes at ages 1-15, 20, 25 and 30 days.

Results: In most categories, when the data were plotted to a third order polynomial regression, there were increases up to ca. 12 days for both sexes except for sound pressure level. In this category males reached a peak at ca. 18 days. Plotted points between 8-16 days for both sexes were very similar, and also within this time period we noticed the best response in bioassays of responses to sound [see report 43 73(1-6)].

Plans: With these data and our ability to identify behavior patterns (flight, aggression, signalling, etc.) by analyzing sound production as background information, we will evaluate the use of sound analysis as a measure of quality. Flies selected through rearing, with various nutritional or rearing practice histories, or subjected to various treatments such as irradiation or marking will be compared through sound analysis and potential detrimental effects identified.



Sound Production by Conotrachelus nenuphar: I. Morphological  
Description of Sound Producing Structures of  
Males and Females

T. Carlysle, C. Calkins and J. C. Webb

Objectives: The location and description of all potential stridulatory areas of plum curculios with special emphasis on sexual dimorphism.

Methods: We will examine the body surface of plum curculio adults with dark phase and scanning electron microscopes to detect sites of potential sound producing structures. Detailed descriptions, measurements, drawings, and photographs of these structures and comparisons of morphological differences existing between males and females will be made.

Results: File-like structures have been located and identified from the ventral surface of the posterior portion of the elytra. These structures are sexually dimorphic indicating that the sound produced by each sex is different. Sound is produced by the flexion of the abdomen resulting in the pygidium (which has a comb-like structure), striking across the files of the elytra. Other structures have been tentatively located in the coxal sockets but have not yet been examined by the scanning electron microscope.

Plans: Detailed examinations of the entire body surface will be made with the scanning electron microscope. Since different sounds are apparently produced by each sex, stridulation may be involved in sexual attraction or mating behavior. Before experiments characterizing these sounds can be completed, the sites of sound production must be identified.





Sound Production by Conotrachelus nenuphar:  
II. Characterization and Reproduction of  
Sounds Produced in Response to Various Stimuli

J. C. Webb and C. Calkins

Objectives: To identify and characterize the different sounds produced by adult plum curculios; to ascertain the conditions necessary for the production of different types of sound.

Methods: The proper environmental conditions (temperature, light intensity, humidity, etc.) and stimuli necessary to elicit sound production will be determined. Sounds will be recorded using a condenser microphone located in an anechoic chamber. This microphone will feed into a sound pressure level meter, then into a wide band data tape recorder where it will be stored for analysis. If necessary, the stored data will be transferred to a bin loop machine where time rating is possible, which will allow better resolution of the analyzed data. Sounds will then be reproduced to determine reactions of beetles to various intensities and frequencies.

Results: Certain conditions were observed to stimulate adult beetles to stridulate: exposure to bright sunlight while in petri dishes, near lethal high temperatures, carbon dioxide concentrations at sub-anesthetical levels, grasping of tarsi, legs or other body parts. At least 2 separate sounds were emitted when beetles were congregated in groups; their significance was not ascertained.

Plans: Continued efforts will be made to identify different sounds and the conditions necessary to produce each type. Sexes will be isolated to stimulate calling behavior. Sounds will be reproduced and played back to observe behavioral responses. If sound production is involved with mating behavior, recordings will be tested to see if mating behavior can be disrupted.



Sound Production by Conotrachelus nenuphar: III. Predatory  
Inhibition by Stridulation

C. Calkins, J. C. Webb and others

Objectives: To determine whether stridulation by adult plum curculios has an inhibitory effect upon predatory organisms.

Methods: The response of predators to plum curculio adults will be tested in observation chambers. Those predators most apt to prey on beetles are: wolf spiders, carabid beetles, frogs, toads, lizards, and birds. Predators which exhibit interest in these beetles as a source of food will be selected for detailed experiments. They will be offered dead beetles, beetles whose stridulatory mechanisms have been rendered nonfunctional, and intact healthy beetles, and feeding responses recorded. Sounds produced by intact beetles under attack will be recorded to determine if stridulation is actually the mechanism of protection.

Results: Adult beetles were exposed to wolf spiders in clear plastic containers. All spiders reacted to the beetles by attacking and attempting to consume them. They were not successful in eating intact, living beetles but had no hesitancy in consuming dead beetles.

Plans: Contacts with herpetologists, entomologists, and ornithologists will be made for identification of organisms and for access and use of their caged living specimens.



Binding of the Sex Attractant of the Almond  
and Indian Meal Moth to Antennal Proteins  
In vitro

S. M. Ferkovich, L. L. Sower, and R. R. Rutter

Objective: To develop information on the mechanism of insect olfaction through studies of proteins in the antennae of the almond moth, Cadra cautella (Walker), and Indian meal moth, Plodia interpunctella (Hübner), which may function in perception of the sex attractant and related synergists and inhibitors.

Methods: Antennae were homogenized in Tris-HCl buffered sucrose (.5M), pH7.5 and centrifuged at 20,000 g for 45 min and the supernatant analyzed on 7% polyacrylamide gels. The pheromone, (Z-E)-9,12-tetradecadien-1-ol acetate (50 µg) was preincubated with the supernatant (200 µg protein) prior to electrophoresis to determine whether pheromone-binding would induce specific structural changes in certain proteins (receptors) which might be reflected as alterations in Rf values, staining intensity (sites available for stain, Coomassie blue, on pheromone-bound protein), number of subunits, etc. Pheromone degradation was monitored by extraction of the pheromone (or metabolite) from the gel and gas chromatography.

Results: Twenty-three bands in the male and 15 in the females of Plodia and 24 bands in the male and 22 in the female of Cadra were detected. Preincubation with pheromone altered the intensity of certain bands in both sexes of Plodia and Cadra, indicating that the pheromone-binding blocked sites on the proteins which also bind the Coomassie dye (reacts with  $\text{NH}_3^+$  and nonpolar protein groups). However, these results were not precisely repeatable, possibly because of the unpredictable stoichiometry of the dye.

In a preliminary experiment using male Plodia antennal protein, it was found that several slow to moderately fast running esterases extracted from gels degraded the pheromone to the corresponding cis, trans alcohol.

When the cis, trans alcohol, an inhibitor in Cadra, was preincubated with the antennal protein from females and males and then analyzed by disc gel electrophoresis and gas chromatography, 4.7 and 11.3% of the alcohol, respectively, was bound to a slow running high mol. wt. band.

Plans: The alcohol binding protein(s) will be characterized, and the dissociation constant will be determined and compared with analogs of the alcohol. In addition, Kms of esterases degrading the pheromone will be determined.



Insect Antennal Esterases: Initial Purification  
and Absolute Specificity

M. S. Mayer, S. M. Ferkovich and R. R. Rutter

Objectives: To ascertain if esterase activity in soluble antennal proteins is an integral part of the transducing process, more data are needed to determine: (1) How many esterases are present; and (2) If the specificity of 1 or more of these parallels behaviorally and electrophysiologically determined specificities.

Methods: Sonicated, soluble antennal proteins were subjected to gel filtration followed by reaction of pheromone and isomers with single fractions. Fractions of the two active regions (I and II) revealed by the above experiment were combined and reacted with the pheromone and 5 isomers of the pheromone.

Results: Gel filtration revealed at least 2 active peaks of esterase activity (Fig. 1). Individual fractions indicated that the pheromone was preferentially degraded over the E-isomer and the saturated homolog of the pheromone. However, when the fractions of the active regions were combined and reacted 18 hrs with the pheromone and 5 isomers or homologs, the pheromone was less actively degraded than the other chemicals. Esterase activity was also observed from female antennae and from the legs of males (Table 1).

Plans: To continue efforts to isolate a single esterase with high specificity for pheromone.





Table 1. Relative percentage isomeric alcohol<sup>1/</sup> produced from overnight incubation with Group I (combined fractions (10-18)) and Group II (combined fractions (20-28)) from male and female antennae and male legs

Percent Conversion of Acetate to Isomeric Alcohol By:

Chemical	Male Antenna Fraction		Female Antenna Fraction		Male Legs Fraction	
	I	II	I	II	I	II
(Z)-7-dodecen-1-ol Acetate	51.5	56.9	54.6	45.8	5.3	27.5
(Z)-8-dodecen-1-ol Acetate	99.2	92.9	97.6	89.5	25.3	55.8
(Z)-9-dodecen-1-ol Acetate	39.0	74.7	59.6	40.7	1.6	8.7
(E)-7-dodecen-1-ol Acetate	90.9	31.4	69.4	—	4.2	21.4
(Z)-7-dodecen-1-ol Formate	77.1	43.5	98.9	99.7	33.6	91.6
Dodecan-1-ol Acetate	60.8	49.2	34.7	25.8	3.1	11.7

<sup>1/</sup> (Z)-7-dodecen-1-ol was confirmed as the reaction product from (Z)-7-dodecen-1-ol acetate by comparing retention times on two GLC columns and mass spectrometry.



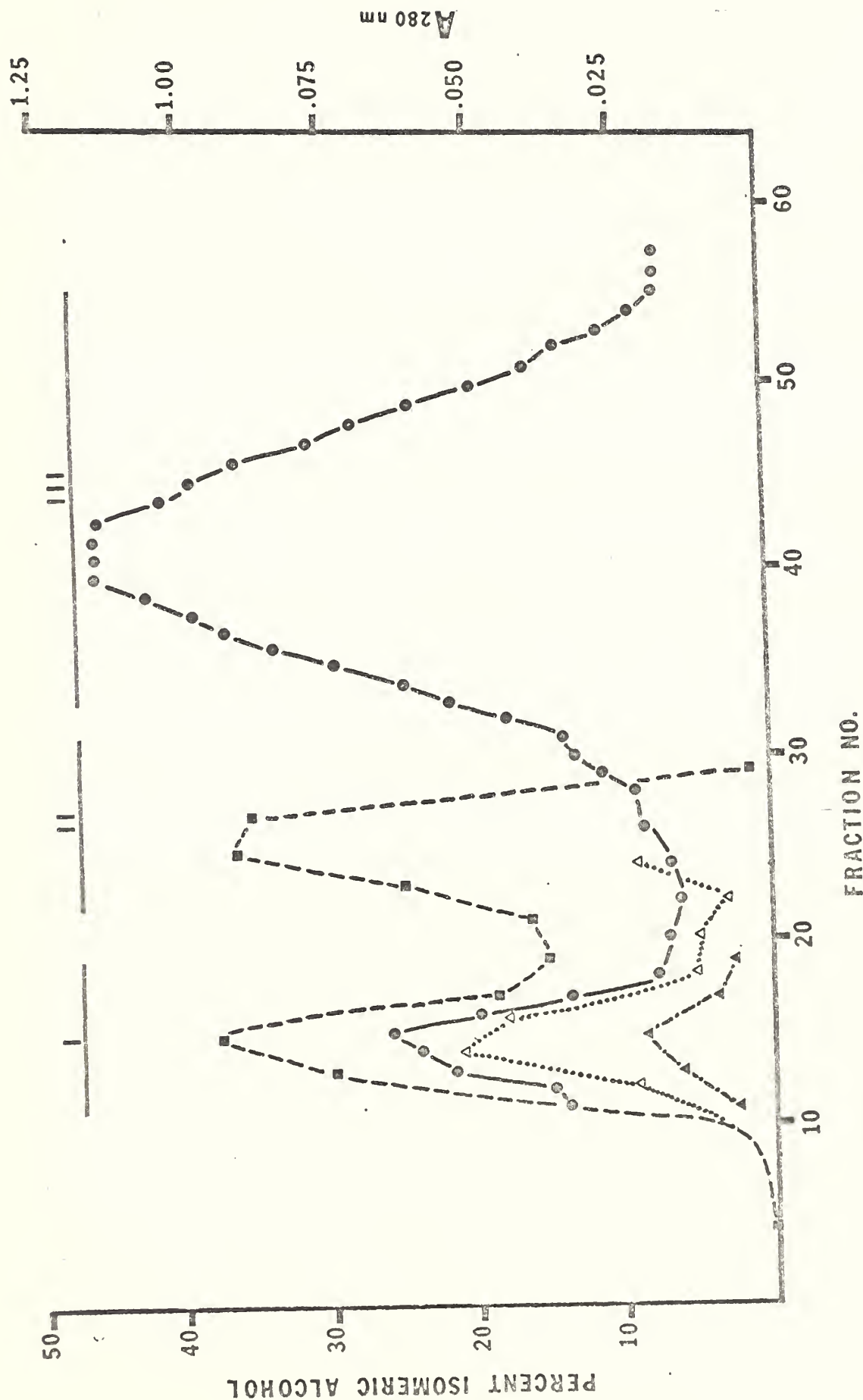


Fig. 1. Elution pattern of soluble male antennal proteins (370 pairs) from a Sephadex G-200 column equilibrated with 0.05 M Tris-HCl, pH 7.5 (right-hand ordinate, filled circles). Reaction of (a) Z-7-dodecen-1-ol acetate, filled squares; (b) (E)-7-dodecen-1-ol acetate, filled triangles; (c) dodecan-1-ol acetate, open triangles.



Electrophysiology of Single  
Unit Pheromone Reception

M. S. Mayer

Objectives: To determine the qualitative range of responses by single unit pheromone receptors in the cabbage looper, Trichoplusia ni (Hübner), and to correlate the quantitative range with attraction. This information would provide critical assessment of the absolute effective range of a pheromone under field conditions.

Methods: Standard recording techniques employing uninsulated tungsten electrodes placed near the sensilla trichodea by micro-manipulators were used. Pheromone was dispensed from glass tubes used in olfactometric studies.

Results: Incomplete data indicate a range of single unit responses whose threshold is extremely close to the threshold of attraction. A second unit in the same sensilla responds to Z-7-dodecen-1-ol but not to the pheromone, Z-7-dodecen-1-ol acetate. Other sensilla types have been characterized according to their responses during the course of this study.

Plans: To continue collecting quantitative data on pheromone and inhibitor response and to correlate these data with attraction. Other ancillary information on olfaction will be developed.



Studies of the Electroantennogram  
of the Cabbage Looper,  
Trichoplusia ni (Hübner)

H. S. Mayer and J. D. James

Objectives: Because many pheromone identifications are being confirmed in part by electroantennogram (EAG) assay, more details of this technique must be studied prior to its general acceptability for this purpose. The cabbage looper, whose behavior to a large range of chemicals is already known, is one of the best choices for this purpose.

Methods: We are using standard recording techniques employing glass Ag-AgCl micro-pipette electrodes. The stimulus is delivered by injecting known concentrations of head-space volume into an airstream constantly impinging on the antenna.

Results: Few qualitative differences in the EAG of the pheromone and 50 other chemicals were observed. Some slight differences in the qualitative aspects of the EAG may be present in various saturated ester analogs of the pheromone; however, further studies are required.

Plans: To continue these studies with the objective of detailing potential problems in using the EAG as an assay for pheromone identification.





Studies on the Infrared Emission of Pheromones

P. S. Callahan

Objective: In order to understand how the energies from insect sex attractants and host plant odors couple to the insect antennae, the infrared emission of the pheromone and scents must be known. The project will locate and plot those emissions.

Methods: A fourier analysis spectrophotometer was modified to utilize a newer powerful "glowbar" source and a special chamber constructed over the IR detector so the pheromone was enclosed in total darkness.

Results: Preliminary examination of the cabbage looper, Trichoplusia ni (Hübner), pheromone in the 7 to 22 micron region showed strong absorption and maser-like emission lines in the IR windows as predicted.

Plans: Further work is in progress irradiating the molecule with various wavelengths of visible UV and near IR light in order to study the interaction of the molecule with short wavelength frequencies.



Electrical Charge on the Antenna of Cabbage Looper,  
Trichoplusia ni (Hübner)

W. K. Turner and P. S. Callahan

Objective: To measure the electrical charge naturally existing on cabbage looper antennae and the maximum charge that can be placed on the antenna. The larger objective is to determine electrical characteristics of the antenna and relate these characteristics to the insect's electromagnetic environment with regard to communication and host location.

Methods: Three methods were used to determine the maximum net charge that a cabbage looper antenna will accept. Charge was placed on the antenna by touching it to a flat surface maintained at  $\pm 500$  volts dc. The net charge was determined either with a commercial electrometer, by "floating" the antenna in the electric field between capacitor plates, or by use of a quartz - fiber torsion balance and plate-capacitor combination. Also, antennae were removed from insects with insulated tweezers and the charge measured with the electrometer.

Results: Quantitative measurements by the three methods were close, varying by much less than an order of magnitude. The values of maximum charge were approximately  $3 \times 10^{-2}$  statcoulombs (1 statcoulomb =  $2.08 \times 10^{-9}$  unit charges =  $3.335 \times 10^{-10}$  coulombs). The antenna could be given a positive or negative net charge. The charge on antennae removed from the insects with insulated tweezers varied from  $10^{-4}$  to  $5 \times 10^{-4}$  statcoulombs, always positive.

Plans: This project is temporarily suspended until measurement methodology is decided upon.



## Electrical Measurement of Dielectric Wax

P. S. Callahan and R. Mankin

Objective: In order to understand the detection capabilities of the insect exoskeleton a better understanding of insect waxes is necessary. This project will measure the electrical properties of bees wax.

Methods: A layer of purified bees wax was coated on a 3" x 3" metal electrode surface. The thickness of the wax was measured with a scanning electron microscope. Measurements of the homo and hetero charges on a charged thickness of wax were taken with a Kiethly electrometer.

Results: Studies on the electret properties of pure bees wax indicate that a 3" x 3" square one mm thick will hold an electret charge of over 150 mV. The charge shorted to ground trickled off in approximately two days. As predicted, the charge was a hetero charge.

Plans: Further work is in progress to construct a system for measuring hetero and homo charges on layers in the micro range of thicknesses. The charged waxes will be irradiated with short wavelengths in order to study the electrical charges affected by radiation.



Studies on the Evolution of Insect Antennae

P. S. Callahan and T. Carlysle

Objective: To understand the evolutionary development of the insect antennae.

Methods: The antennae of representative species of 22 orders of insects are being studied with the scanning electron microscope.

Results: Scanning photographs have been obtained of the antennae of 28 species in 18 different orders. Antennae are being compared from order to order for complexity and array characteristics.

Plans: When all of the photographs are completed the evolutionary development of the antennae sensors will be compared between the insects of the primitive orders and the more advanced orders.





Comparative Morphology of the Ovipositor Sensilla  
of Parasitic Wasps of Differing Habits

P. Greany and S. Hawke<sup>1/</sup>

Objectives: To examine the sensilla in the ovipositors of a number of parasites of varying habits and sizes to determine whether any general statements could be made as to the appearance, diversity, dispersion, and number of sensilla which could in turn be correlated with the specific characteristics of the parasites. The following species of parasites were chosen for study: Opius longicaudatus (Ashm.), Parachasma cereum (Gahan), Devorgilla (Nemeritis) canescens (Grav.), Bracon hebetor Say, and Trichogramma evanescens Westwood.

Methods: Scanning and transmission electron microscopy studies have been conducted on each species. The scanning EM was performed by coating the specimens with a thin layer of carbon, then gold, and examining them at 10 kV. Transmission electron microscopy involved cutting the ovipositor off very near the tip in either isosmotic 4% glutaraldehyde or Karnovsky's paraformaldehyde fixative. The conditions of fixation are not yet entirely satisfactory, and the results are not yet optimized. The tissue was then post-fixed in osmium tetroxide and dehydrated through a graded acetone series before infiltration and embedment in Spurr's low viscosity embedding medium. Sections were cut using a diamond knife and a Sorvall Porter-Blum MT-2 ultramicrotome. Sections were stained with uranyl acetate and lead citrate before examination.

Results: Scanning EM has revealed the presence of sensilla in the ovipositors of each species. Generally these either had the conformation of a shallow depression about 2 microns in diameter with a flat non-porous central region or a shallow depression about the same size but with a dome or peg in the center. In some cases, pores could be discerned at the apex of the dome, and longitudinal ridges on the pegs were like those on the pegs of antennal sensilla coeloconica. In Trichogramma, only the medial ovipositor stylet appeared to bear sensilla; all other species possessed sensilla on the lateral stylets as well. Though incomplete, transmission electron microscopy results revealed that generally there are sensilla innervated by single bipolar neurons and multicellular sensilla present in the ovipositors of each species. Additional work is needed to be able to fully correlate the scanning and transmission EM results.

Plans: To improve the treatment of the tissue during preparation for transmission electron microscopy. Efforts will then be made to fully describe the morphology of each type of sensilla so that comparisons can be made on the various types of parasites selected, which include large and small wasps, ecto- and endoparasites, and larval and egg parasites.

<sup>1/</sup> Assistant Professor, Department of Biology, Willamette University, Salem, Oregon.



Reproductive Biology of the Velvetbean Caterpillar,  
Anticarsia gemmatalis Hübner

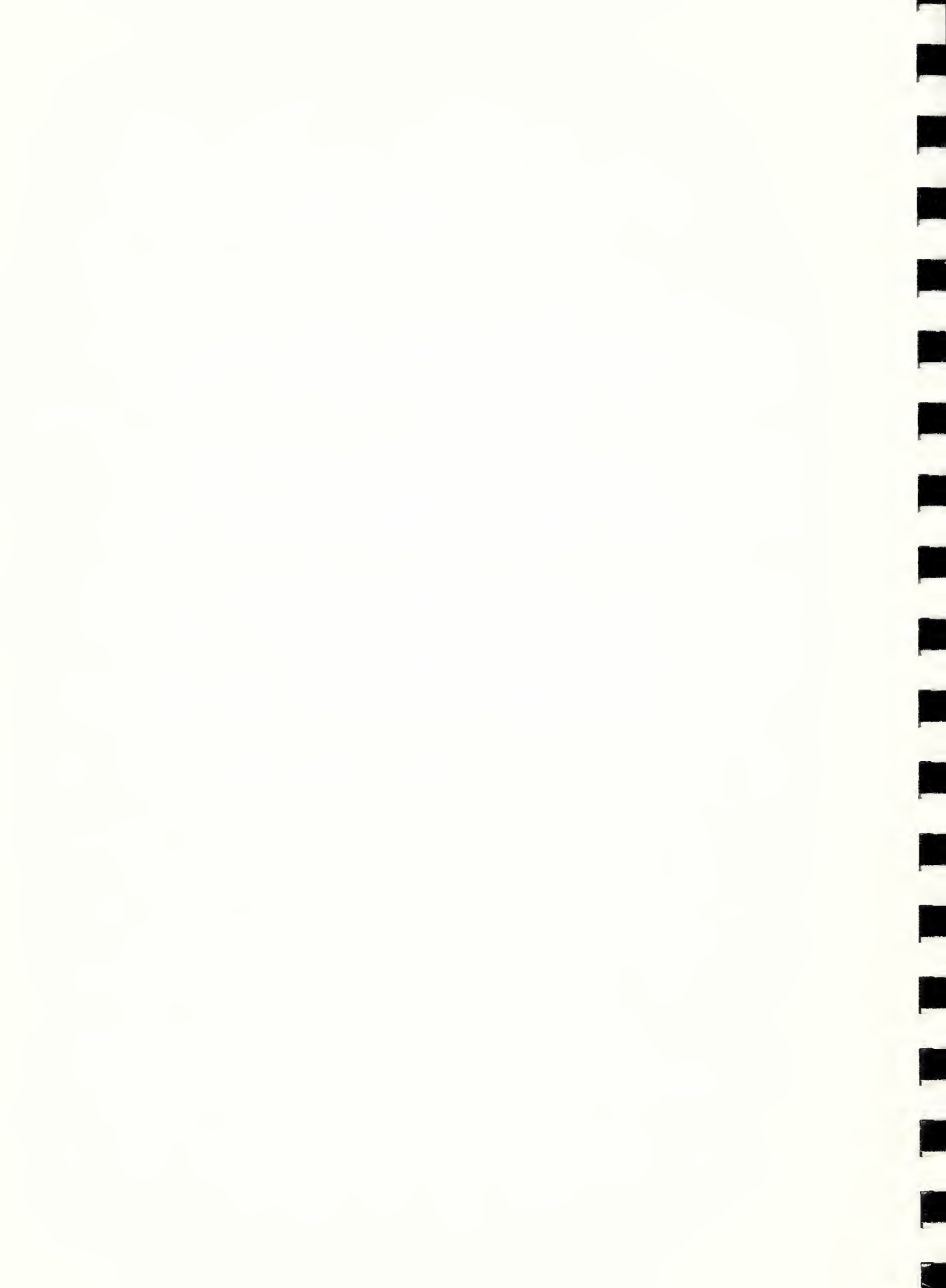
N. C. Leppla and T. C. Carlisle

Objectives: To determine the reproductive potential, pattern of adult maturation, courtship behavior, and the influences of temperature and humidity on reproduction in the velvetbean caterpillar, whose reproductive biology is little known, although it has become a major pest of soybeans.

Methods: Mating behavior is being studied by direct observation and with an actograph. Patterns of maturation and reproductive potentials are being determined by pairing appropriate densities and ages of moths. Histological analysis will support this data. The influence of some environmental factors has been documented.

Results: Velvetbean caterpillar moths are typical noctuids, being continuously active at night. Mating occurs primarily during the second half of the scotophase, and is initiated by "calling" females. The females have never exhibited more than a single mating, while the potential for males is unknown. Spermatophore transfer requires ca. 70-80 min. Maturation of a population requires 8-12 days, and 85% plus RH is required for high levels of reproductivity.

Plans: This study will be completed as outlined during FY 1974.



Reproductive Systems of the Lovebug, Plecia nearctica

N. C. Leppla and T. C. Carlisle

Objectives: The Bibionidae possess a unique reproductive system which has not been described. In this study, male and female reproductive systems will be dissected and analyzed histologically. This information will be related to the mating behavior and reproduction of this insect.

Methods: Males, females, and coupled pairs of lovebugs have been fixed and embadded in paraffin for histological examination. Also, samples collected from the field have been maintained in the laboratory for biological studies and preserved for dissection. Mating frequency, method of coupling, mechanics of sperm transfer, and associated internal morphology will be documented.

Results: Apparently, female flies mate a maximum of 2 times, once on each side of a complex system. With females which have already mated once, males must determine the empty side, align properly, and maintain coition for extended periods. The spermatophores are very compact and appear to be fitted specifically to the females.

Plans: This very brief study will be completed during FY 1974.



Periodicity of Plum Curculio Activities

C. Calkins, N. Leppla, K. Turner, and E. Hamilton

Objectives: To determine and understand the diel activity rhythms of the plum curculio, Conotrachelus nenuphar, in the laboratory and in the field.

Methods: Laboratory reared plum curculio adults were tested with an actograph apparatus in groups of 10 in a 12 hour photophase for 72-hour periods. Periodicity of respiration was determined in a respiration chamber with a 12 hour photophase with increasing and decreasing light intensities. Additional tests involving different combinations of age, sex, seasonal appearance, etc. will be used.

Results: Adults exhibited a definite rise in activity beginning about 3 hours after onset of darkness which was maintained throughout the remaining scotophase and suddenly intensified for one hour with the onset of the photophase. Thereafter, activity diminished quite suddenly and almost ceased throughout the light phase. Respiration activity followed the same pattern with the exception that it did not intensify with the onset of the light period. Because light intensity increased gradually in these tests, a conditioning effect may have been produced. These preliminary tests indicate that this species may be nocturnal in its activity.

Plans: This study is being conducted in 2 phases; one involving laboratory cultures and the other involving field populations. The former will give insight on how and when laboratory behavior studies should be conducted. The latter may reveal when mating, dispersal, migration, and oviposition occur.





The Flight Ability of the Plum Curculio in the Laboratory

J. L. Sharp, C. O. Calkins, and D. R. Bennett

Objective: To study the flight performance of the plum curculio, Conotrachelus nenuphar (Herbst) in the laboratory.

Methods: Adult plum curculios were attached to flight mills to determine if the system could be used to study curculio flight ability.

Results: Observations indicated that the mill system could be used to study the flight ability of the curculios. Also, preliminary tests showed that the curculios periodically fly for short distances, followed by rests.

Plans: A comprehensive flight study is planned to determine the effects of sex, age, and other factors on locomotor capability.



## The Flight Ability of Caribbean Fruit Flies in the Laboratory

J. L. Sharp, D. R. Bennett, and D. L. Chambers

Objectives: To study the flight ability of the Caribbean fruit fly, *Anastrepha suspensa* (Loew), and the effects thereon of various conditions, treatments, and genetic selections.

Methods: Male Caribbean fruit flies were fastened to a flight mill system and allowed to fly for several hours. Flight categories investigated were distances flown, velocity, duration, and frequency of flights.

Results: Preliminary test results with 8-day-old male flies are shown in Table 1.

Plans: Flight ability, as an expression of vigor, will be evaluated as a test parameter for measuring the quality of insects selected, mass reared, and treated for use in control programs such as sterile release.

Table 1. Mean flight data  $\pm$ SE of 8-day-old male Caribbean fruit flies for 2.2 hrs.

Flight Categories	Mean $\pm$ SE
Distances flown (m)	1750 $\pm$ 5.5
Sustained velocity (m/sec)	0.50 $\pm$ 0.05
Velocity, including stops (m/sec)	0.22 $\pm$ 0.06
Peak velocity (m/sec)	0.58 $\pm$ 0.05
% of time flying	41.3 $\pm$ 11.4
No. flights/hr	9.6 $\pm$ 3.5



The Circadian Periodicity of Activity of Caribbean Fruit Flies

J. L. Sharp, N. C. Leppla, and D. R. Bennett

Objective: The objective of this study is to determine the circadian periodicity of the activity of the Caribbean fruit fly, Anastrepha suspensa (Loew).

Methods: Actograph studies monitored the periodicity of activity of Caribbean fruit flies in tests conducted with wild flies allowed to emerge in the laboratory. Emergent adults from field collected rose apple, Syzygium jambos (L) Alst., taken from Redland Spice Park, Florida, were provided yeast hydrolysate, sugar, and water and were maintained in 30.5 x 30.5 x 30.5-cm cages at 28°C, 60% RH, and 315 ft-c in an environmental chamber. Fifty flies of each sex at 16 days of age were transferred from the holding cages to actograph cages for 72 hrs and provided with food and water. Studies were done with these flies at 27°C, 65% RH and 200 ft-c of light with a light:dark cycle of 14:10 (light 6 am-8 pm). Data were analyzed from the central 24 hr period.

Results: Both sexes are diurnal. Their activity ceased as soon as it was dark and increased when the lights were turned on. Males are most active between 4-8 pm, the same time when maximum mating seems to occur. Females, less active than males, are most active between 6-8 pm.

Plans: Simultaneous measurements of wild flies and laboratory-reared flies will be carried out from adult emergence for much longer periods under varied light, temperature, and RH regimes.



Effect of Increasing Levels of Gamma Irradiation  
on Wingbeat Frequencies of the Caribbean Fruit Fly

J. L. Sharp, D. R. Bennett, and B. J. Smittle<sup>1/</sup>

Objectives: We can obtain a reliable indication of the effects of gamma irradiation on flight ability by directly comparing the wingbeat frequencies of irradiated versus nonirradiated flies. This report furnishes preliminary data of gamma irradiation effects on wingbeat frequencies of male and female Anastrepha suspensa (Loew).

Methods: Pupae were irradiated in normal air with dosages of 2.5, 5.0, 10.0, and 20.0 kilorads (Kr) 1-2 days before adult eclosion. Wingbeat frequencies at different ages were compared to nonirradiated flies for both sexes.

Results: Table 1 shows the mean wingbeat frequencies for both sexes at different dosages and ages. No statistical comparisons have been conducted; however, a few trends are apparent. Wingbeat frequencies increase with age for both sexes. Dosages up to 5.0 Kr seem to increase frequencies in both sexes and 20.0 Kr reduces them.

Plans: An additional replication will be carried out before statistical comparisons are conducted and conclusions derived.

<sup>1/</sup> Insects Affecting Man Research Laboratory, Gainesville, Fla.





Table 1. The mean wingbeat frequencies  $\times 10^3$  (cycles/min) of males and females at different dosages and ages. 1/, 2/

Dosage(Kr)	Age in Days								
	1	4	7	10	13	16	19	22	25
0.0	$\frac{6.2}{6.6}$	$\frac{8.7}{8.4}$	$\frac{8.5}{8.3}$	$\frac{8.3}{8.9}$	$\frac{8.8}{9.2}$	$\frac{8.7}{8.7}$	$\frac{9.9}{9.6}$	$\frac{8.7}{9.0}$	$\frac{9.3}{9.5}$
2.5	$\frac{6.5}{6.8}$	$\frac{8.8}{8.6}$	$\frac{8.6}{8.6}$	$\frac{8.7}{8.7}$	$\frac{8.9}{9.1}$	$\frac{8.2}{8.4}$	$\frac{8.6}{9.4}$	$\frac{8.7}{8.3}$	$\frac{9.3}{9.1}$
5.0	$\frac{6.8}{7.0}$	$\frac{8.5}{8.7}$	$\frac{8.5}{8.9}$	$\frac{8.8}{9.0}$	$\frac{8.9}{9.0}$	$\frac{8.2}{8.6}$	$\frac{8.7}{8.9}$	$\frac{8.4}{8.6}$	$\frac{8.9}{9.9}$
10.0	$\frac{6.7}{6.5}$	$\frac{8.3}{8.4}$	$\frac{8.3}{8.5}$	$\frac{8.5}{9.0}$	$\frac{8.7}{9.0}$	$\frac{0}{8.4}$	$\frac{0}{8.7}$	$\frac{0}{8.9}$	$\frac{0}{9.9}$
20.0	$\frac{6.5}{6.7}$	$\frac{7.6}{8.1}$	$\frac{0}{8.2}$	$\frac{0}{8.4}$	$\frac{0}{8.6}$	$\frac{0}{0}$	$\frac{0}{0}$	$\frac{0}{0}$	$\frac{0}{0}$

1/ Each mean ( $\sigma^2 / \sigma^2$ ) based on 2 reps, 12  $\sigma^2$  and 12  $\sigma^2$ /rep. Means for males are above, females below.

2/ 0 indicates no data collected due to excessive mortality.



Effects of Gamma Irradiation on the Longevity and Fecundity  
of Male and Female Anastrepha suspensa (Loew), Fruit Flies

J. L. Sharp, B. J. Smittle<sup>1/</sup>, and D. R. Bennett

Objective: To determine the effects of gamma irradiation on the longevity and fecundity of Caribbean fruit fly adults.

Methods: Pupae 1-2 days before adult eclosion were gamma irradiated in groups of 50 ml with 2.5, 5.0, 10.0, or 20.0 kilorads (Kr) in normal air. Another group of pupae, not irradiated, was used as a control. Emergent adults were kept in an environmental chamber at 29°C, 60% RH, 315 ft-c of light, and a 13:11 hr L:D cycle. Dead adults were aspirated daily from the cages and counted. The numbers were converted to percentages and the resulting survival rates were determined.

Results: The sex ratio of males and females and percent emergence are given in Table 1. Table 2 shows the time in days when 50 and 95% of the population are dead. These preliminary data show that adult fruit flies have a long life span under normal laboratory conditions and that irradiation at high levels severely affects longevity.

1/ Insects Affecting Man Research Laboratory, Gainesville, Fla.



Table 1. Percent emergence and sex ratios of Caribbean fruit flies

<u>Dosage (Kr)</u>	<u>% Emergence</u>	<u>Sex Ratio (<math>\sigma^7</math>:<math>\pm</math>)</u>
0	51.9	1.6:1
2.5	50.7	1.8:1
5.0	52.8	1.6:1
10.0	49.7	1.6:1
20.0	43.4	1.3:1

Table 2. Time in days when 50 and 95% of the population are dead

<u>Dosage (Kr)</u>	<u>50%</u>		<u>95%</u>	
	<u><math>\sigma^7</math></u>	<u><math>\pm</math></u>	<u><math>\sigma^7</math></u>	<u><math>\pm</math></u>
0	32	28	88	65
2.5	30	37	73	75
5.0	19	21	57	57
10.0	12	22	37	63
20.0	8	17	15	34



Demonstration of the Sexual Attractiveness of Lab-Reared  
Plum Curculios, Conotrachelus nenuphar  
to Wild Populations

C. Calkins and A. Hill

Objectives: To determine if sexual attraction can be demonstrated in plum curculio populations.

Methods: Well ventilated chambers containing virgin and mated male and female plum curculios in various combinations were suspended inside cylinders of hardware cloth dipped in Tack Trap<sup>®</sup>. These apparatus were suspended 4-5 ft. above ground in trees in 3 sites in Alachua County: at Hawthorne in a peach-nectarine orchard; in plum trees in southeast Gainesville; and in a wild plum thicket 10 miles west of Gainesville. All insects caught on the treated hardware cloth were placed in labeled vials and brought to the laboratory for identification. Tests were begun in February and are still continuing.

Results: Four plum curculios were trapped west of Gainesville, while 2 were caught in southeast Gainesville. The orchard at Hawthorne was sprayed on a weekly basis and curculio populations were probably very low or nonexistent. There appeared to be no set pattern of attraction. However, these very preliminary data suggest the possibility that there is an aggregating response.

Plans: This study will be intensified in many more locations next year. Fruit will be added to the containers to see if attraction depends upon the presence of food or oviposition sites.





Host-finding Behavior of Opius longicaudatus (Ashm.)

P. D. Greany, D. L. Chambers, J. H. Tumlinson, and D. R. Sukkestad

Objectives: To define the mechanisms used in host-finding by females of the braconid parasite, Opius longicaudatus (Ashm.). Preliminary observations indicate that the laboratory host, the Caribbean fruit fly, Anastrepha suspensa (Loew), produces a kairomone which heightens searching behavior in female parasites. Efforts are being made to define and optimize the biotic and abiotic factors relating to the host-searching response of female parasites. Isolation and chemical identification of the kairomone is sought.

Methods: To determine the influence of mating and age of parasite females upon host selection, ten newly-emerged virgin females and 9 male-female pairs were placed in oviposition units (Fig. 1) with 50 fully-grown host larvae. Hosts were replaced each day and held for parasite emergence. Proof of mating was established by the presence of female progeny among the offspring of mated females.

To determine effect of the stage of development of the host and composition of the host's diet upon attractiveness to parasites, a semi-solid artificial diet is being employed so that larvae can be exposed in situ at any stage desired, and so that host plant products can be incorporated. These studies are not yet complete, but success has been obtained in inducing parasites to accept larvae in the semi-solid diet.

Results: Both mated and unmated females were found to be most responsive at 4 days of age (Figs. 2,3). No profound difference appeared in the oviposition rates of mated and virgin females, producing means of 29.6 and 22.1 progeny, respectively. All mated females produced female progeny, indicating that insemination did occur. Female progeny occurred among the first offspring, indicating that the premating period did not exceed the preoviposition period. Although either mated or virgin females could be used, future bioassays will more easily be conducted using 4-day-old virgin females without prior oviposition experience to optimize the response to host-seeking stimulants.

Plans: To define the preferred stage and technique of exposure of host to achieve the greatest level of response possible when conducting bioassays. Also, to establish the effect of incorporating host plant products into a semi-solid diet upon the host's attractiveness to parasites. Initial efforts toward isolating the kairomone from infested peach juice are also being initiated at this time.



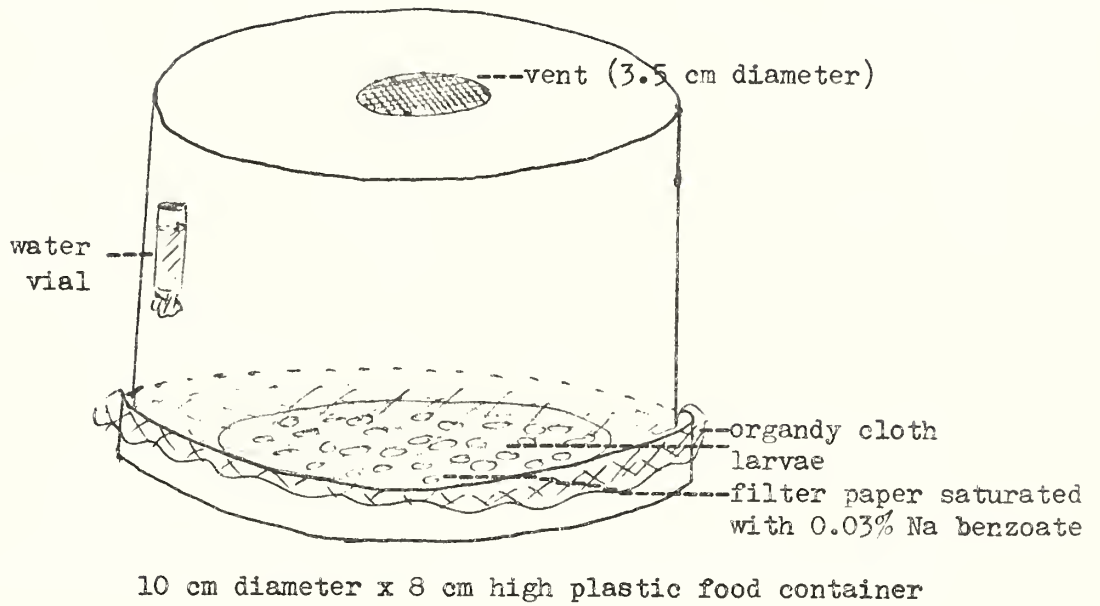


Figure 1. Oviposition unit.



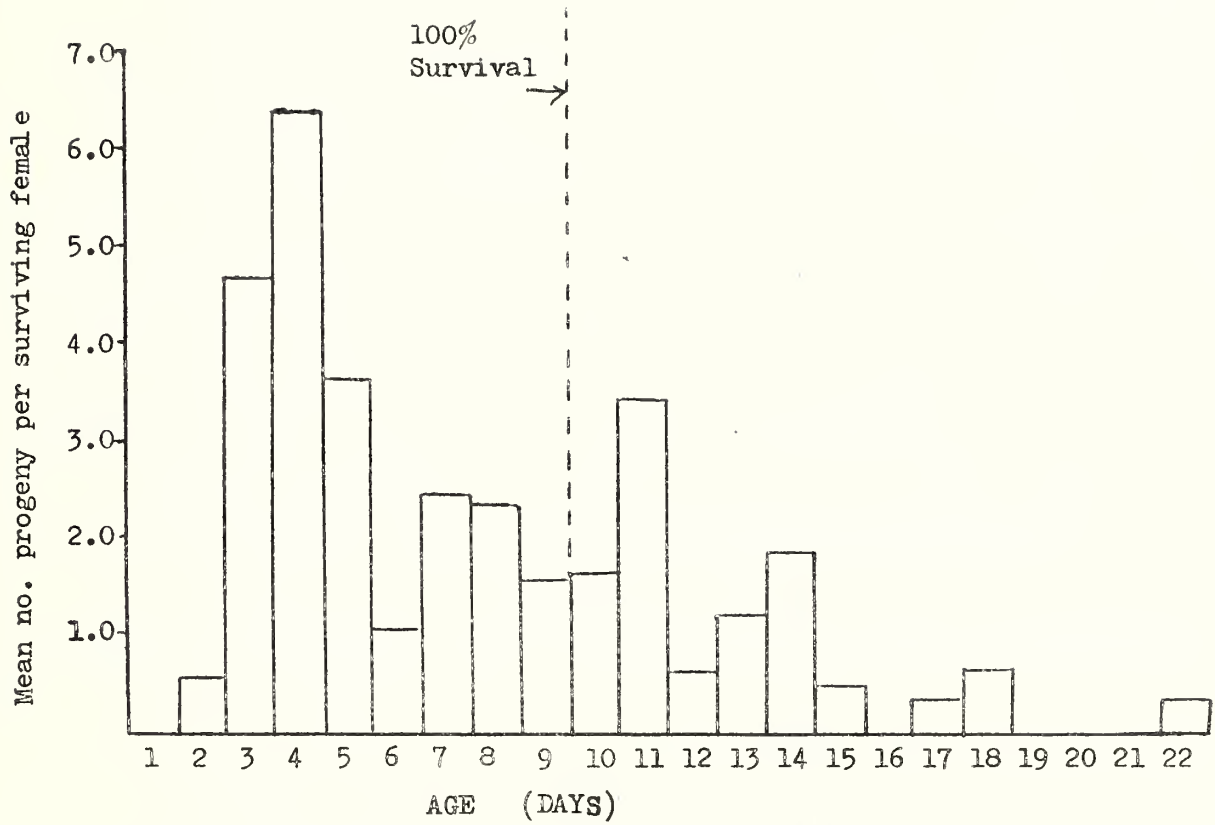


Figure 2. Age-specific responsiveness of mated females to fully-grown host larvae.



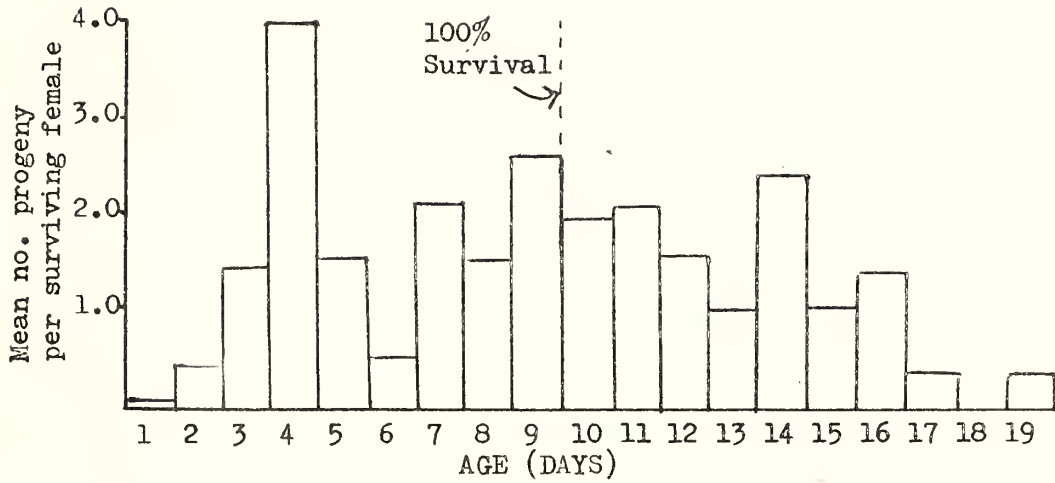


Figure 3. Age-specific responsiveness of virgin females to fully-grown host larvae.





Flight Ability of Trichoplusia ni in the Laboratory

J. L. Sharp, E. R. Mitchell, D. R. Bennett, D. L. Chambers,  
and C. Morgan

Objectives: To determine and compare flight performance of variously selected or treated cabbage looper moths, Trichoplusia ni (Hübner) in the laboratory.

Methods: Females having normal body coloring were tested at 5 days after eclosion against females having white body color. Temperature, relative humidity, and light intensity were 28.9°C, 60%, and ca. 100 ft-c.

Results: These preliminary data show that the normal females flew faster and greater distances (Table 1).

Plans: Several flight mill tests are planned using T. ni to determine the effects of pheromone(s), temperature, RH, age, sex, etc. on flight ability.

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Table 1. Flight performance of T. ni females having normal coloring and females having white body coloring<sup>a/</sup>

Flight Category	Normal Females	White Females
Distance flown (m)	3232 ± 797 <sup>a/</sup>	1104 ± 489
Sustained velocity (m/sec)	0.99 ± 0.15	0.78 ± 0.07
Velocity, including stops (m/sec)	0.76 ± 0.18 <sup>a/</sup>	0.28 ± 0.13
Peak velocity (m/sec)	1.08 ± 0.17	0.85 ± 0.08
% Time flying	66.6 ± 13	32.5 ± 14
No. flights/hr	6.78 ± 3	3.97 ± 0.75

<sup>a/</sup> Denotes significant differences at the 5% probability level between means in the same row based on a t-test.



Role of Dispersal and Life History Characteristics in the  
Population Dynamics of the Almond Moth, Cadra cautella, in a  
Citrus Pulp Warehouse

D. W. Hagstrum and Jane Sharp

Objective: This study seeks to identify and describe aspects of the insect's behavior and life history that might be used to improve current methods of control or develop new ones.

Methods: The larval population of the almond moth in a citrus pulp warehouse was sampled weekly using artificial pupation sites. Insects marked with body or eye color mutations were released and recaptured to evaluate the extent of their dispersal within the warehouse.

Results: Preliminary studies have shown that the almond moth completes two generations within the first 17 weeks that citrus pulp is stored, based upon the interval between peaks in population size. There was a 100-fold increase in population size between the 1st and the 2nd generation. The longer the citrus pulp has been stored, the more uniform the distribution of larvae seeking pupation sites becomes. Four nonallelic body and eye color mutations (8 identifiable phenotypic combinations) have been isolated and propagated for use in future multiple release-recapture studies. Preliminary studies indicate that larvae crawl  $\geq 7$  feet in search of a pupation site and that adults fly  $\geq 40$  feet within a day.

Plans: Studies on population dynamics will be continued and more extensive studies on the dispersal of larvae and adults will begin next season.



Role of Constant and Fluctuating Temperatures in  
Determining the Behavior and Development Time of  
Stored-product Insects

D. W. Hagstrum

Objectives: Differences in an insect's response to constant and fluctuating temperatures are often important to the prediction and manipulation of behavior, life history or physiology. This study seeks to identify and quantitate these differences.

Methods: The development times of the red flour beetle, Tribolium castaneum and rice weevil, Sitophilus oryzae, were determined for 8 populations of 50 or 250-350 insects, respectively, that were exposed to constant temperature. Eight populations of similar numbers were exposed to sinusoidal temperature cycles with 10°C ranges. Mean temperatures of 22.5, 25, 27.5 and 35°C were compared.

Results: The stenothermic species, S. oryzae, showed a smaller difference in its response to the 2 environments than did the eurythermic species, T. castaneum.

Plans: Studies will be expanded to include other species. Further work will be initiated to determine the reason for differences in the response of these insects to the two environments.



Dispersal of the Red Flour Beetle,  
Tribolium castaneum Within Flour

D. W. Hagstrum

Objectives: Knowledge of the behavior of insects infesting a commodity is a prerequisite to the development of better methods of detection or new information on the insect's behavior that might be exploited for purposes of control. The dispersal of insects within the commodity is particularly important.

Methods: Breakdown units consisting of a frame sandwiched between two aluminum sheets to provide a cavity either 10 cm wide x 15 cm high x 0.64 cm deep or 30 cm x 30 cm x 50 cm were used. At the end of the test, one of the aluminum sheets was removed, the flour was divided into cells by a partitioner and the contents of each cell were removed and counted. In addition, autoradiography was used to trace insect movement. Studies included an evaluation of the effect of density, size of environment and temperature upon the dispersal of mated female red flour beetles, Tribolium castaneum. Also, the dispersal of larval instars was studied.

Results: The rate of dispersal increased when the density was increased from 10 to 20 beetles/50 gm of flour. However, for lesser or greater densities the rate of dispersal was not different from that of 10 or 20 beetles. Data on rate of dispersal collected within a large environment were identical to those expected, based upon an extrapolation of data collected previously within a small environment. Preliminary results indicate that the turning frequency, but not the total distance moved, increases with temperature. The rate of dispersal increased with larval age.

Plans: Studies on the effect of temperature upon dispersal will be expanded. A predictive model for the movement of T. castaneum within flour will be developed.





Spatial Distribution of Oviposition by Plum Curculios  
in an Unsprayed Nectarine Orchard

C. Calkins

Objectives: To determine the ovipositional pattern of female plum curculios (*Conotrachelus nenuphar*) in relation to the edge of the orchard, topography, fruit abundance, and hibernation sites.

Methods: When very young fruit is infected by plum curculio larvae, it drops from the tree. Thus, the distribution of fallen fruit should give an indication of the early distribution of ovipositing females. The distribution of dropped fruit was plotted in a 14.6 acre unsprayed 2-year old nectarine orchard on 5-22-73. Fruit or remnants of fruit from the previous 5 weeks were still present and could be identified. Samples of fruit were collected and classified as to the number of feeding scars and the number of larvae emerging.

Results: The distribution of dropped fruit did not appear to be random but instead appeared as an aggregated distribution. Correlations between dropped fruit, fruit abundance, topography and surrounding vegetation have not been completed. The relationships of the number of feeding scars per fruit to the percentage of dropped fruit harboring larvae and to the number of larvae per fruit are shown in the following table:

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No. feeding scars	Percentage of dropped fruit having 1, 2 or 3+ scars	Percentage of dropped fruit with 1, 2 or 3+ scars having mature larvae	No. larvae emerged fruit
1	15	43	0.6
2	19	55	0.8
3 or more	67	94	2.7

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There was a total of 5,061 fruit on the ground in this orchard. Using the figures from the table for calculations, 10,380 larvae emerged to pupate. Of the larvae collected in the laboratory from nectarine samples, 69.7% emerged as adults. The sex ratio was ca. 60:40, male to female. As yet, we have no indication of how many female adults are necessary to produce this number of larvae. If pupation success in the field was similar to that in the lab, there were approximately 3,000 female adults emerging in June from this orchard.

Plans: More intensive observations will be made on a weekly basis next year to determine distribution in time as well as in space.



Distributions and Movements of Insects in Sprayed  
and Unsprayed Peach Orchards

C. Calkins, M. McKoy, and A. Hill

Objective: To observe the distributions of insects in time and space within peach orchards in Fla.

Methods: Unidirectional trap-panels of hardware cloth dipped in Tack Trap<sup>R</sup> were positioned at 2-, 4-, 8-, and 12-foot heights at various positions within an unsprayed and 2 sprayed peach orchards. Insects from each panel were removed and placed in labelled vials for transport to the laboratory for identification. The study commenced at early bloom and will continue until October.

Results: A large volume of insects was collected and they are in the process of being sorted and identified. Early indications show that moths (with exceptions of peachtree borer species) and flies must be excluded from our study because of identification problems. Hemiptera, Diptera, and Coleoptera are the orders represented most plentifully. Coccinellidae and Carabidae are the most plentiful families. Insects affecting peaches which have been recovered in fairly large numbers include stink bugs, tarnished plant bugs, and plum curculios.

Plans: When identifications have been completed, we will decide on which species we will concentrate our studies of distribution and movement. We plan to continue trapping in these orchards one more year.



Life Table Studies of Plum Curculio,  
Conotrachelus nenuphar (Herbst)

C. Calkins, A. Hill and M. McKoy

Objectives: To determine the innate potential for population growth of plum curculio and the mortality factors which are operating on each stage.

Methods: The potential egg-laying capacity of plum curculios from the laboratory colony was determined by dissections and by egg counts using apples as oviposition sites. The number of larvae developing in fruit in the field was determined by taking samples of peaches and nectarines from unsprayed orchards in Fla. Parasite recoveries were made by holding fruit and mature larvae collected from the field for parasite emergence. Other mortality factors (e.g., weather, predators, diseases, etc.) will be determined by observations of field populations and by various artificial infestations in simulated environments.

Results: Dissection was not a satisfactory method for determining egg-laying capacity in this species because the oviposition period extended over such a long period of time. Recovery of larvae from apples does not reveal mortality occurring in the egg and early larval stages. Greatest larval recovery rates were 3.4/day extending over a 10-week period. Tests have not been completed. The number of larvae developing in fruit in the field is shown on Table 1.

Peaches contained more larvae per fruit than nectarines, probably because of their larger size. Peaches, from a well-managed orchard, showing gumming and cat-facing were not infested by plum curculio larvae. Gumming apparently was the result of a genetic weakness in early peaches which is expressed only during prolonged wet weather.

Parasitoids of plum curculio were tentatively identified as Microbracon mellitor, a braconid parasitoid which emerged from larvae while still in the fruit, and Triaspis curculionis, a braconid parasitoid which emerged from soil in which plum curculio larvae had burrowed to pupate.

Plans: Mortality factors operating in each stage of growth of plum curculio populations will be determined. Special emphasis will be devoted to egg-laying capacity in the field and overwintering success for assimilation of a life table for this species.



Table 1.--The average number of plum curculio larvae developing in fruit having 0, 1, 2, and 3 or more feeding scars.

No. feeding scars	Average No. larvae/fruit	Range
<u>Peaches - May 3</u>		
0	0.5	0 - 1
1	2.3	0 - 3
2	1.1	0 - 3
3+	3.5	0 - 8
Severe cat-facing	2.8	1 - 6
<u>Nectarines - May 9</u>		
1	0.6	0 - 2
2	0.8	0 - 2
3+	2.7	0 - 5





Development of Genetically Marked Strains of the  
Plum Curculio, Conotrachelus nenuphar (Herbst)

M. Huettel, C. Calkins, A. Hill, and J. Young

Objective: Strains of the plum curculio genetically marked by rare isoenzyme alleles are being developed for use in field studies of the biology and ecology of this insect.

Methods: Twenty-five single pair matings of plum curculios from the laboratory colony were established and the  $F_1$  progeny reared from green thinning apples. A sample of the progeny from each cross is being analyzed by starch gel electrophoresis for variation at 4 enzyme loci. Those crosses which have been shown to carry rare alleles at one or more of the 4 loci will form the basis for the marked strains. Repeated brother-sister matings will result in a number of inbred lines, marked with one or more rare alleles in homozygous combination, by the  $F_3$  generation. The results of these crosses will be monitored by electrophoresis. Outbred strains will then be produced by crossing the inbred lines.

Results: Twenty-one of the 25 original crosses have produced mature progeny over the past 13 weeks in numbers ranging from 4 to 224. Most crosses are still producing  $F_1$ 's. Mature larvae of the  $F_2$  generation are not expected before August 1, 1973.

Rare alleles have been found at each of the 4 enzyme loci assayed. All appear to be inherited as simple codominant Mendelian characters. The total number of alleles present at each locus is given in Table 1.

Each of the samples of progeny from the 19 crosses assayed to date contain at least one rare allele and so are useful for further breeding purposes. Polymorphic loci for each cross are shown in Table 2.

Plans: Strains marked with rare alleles will be useful for field mark and recapture studies in which the recaptured samples are screened by electrophoresis. Also, local movements within orchards and between host plants may be determined by releasing premated females and subsequently sampling progeny in host fruits. Unmated adults may be released to obtain information about the rate of integration of a laboratory strain into a wild population. The plum curculio may be an excellent insect for these studies because of its low adult population densities in the field.



Table 1. Total number of alleles segregating at four loci in a sample of plum curculios ( $19\sigma^7\sigma^7$  ;  $19\phi^0\phi^0$ ) from the Gainesville lab colony.

Locus	Abbr.	No. Alleles
Alcohol dehydrogenase	ADH	3
Monamine oxidase	MO	2
Esterase-6	EST	2
Isocitrate dehydrogenase	IDH	2

Table 2. Survey of progeny from 19 crosses of plum curculios. Progeny groups carrying one or more rare alleles are indicated by a plus (+). Groups homozygous for a common allele are indicated by a minus (-).

Cross No.	Locus			
	ADH	MO	EST	IDH
1	+	+	+	-
2	-	-	+	+
4	+	+	+	-
5	+	+	+	-
6	-	-	+	+
8	+	-	-	+
10	+	+	+	-
12	-	-	+	+
13	-	-	+	+
14	+	+	-	-
15	-	-	+	-
16	+	+	+	-
18	+	+	+	+
19	-	-	+	+
20	+	+	+	+
22	-	-	+	-
23	-	+	-	+
25	-	-	+	-



Survey of Enzymatic Protein Polymorphisms in the Caribbean  
Fruit Fly, Anastrepha suspensa (Diptera: Tephritidae)

M. Huettel and J. Young

Objective: As background for a study of population genetics of the Caribbean fruit fly, a survey of potentially useful enzyme systems is being carried out.

Methods: Flies reared in the Gainesville laboratory colony have been used for the initial survey. Adults and, in most cases, larvae are electrophoresed using standard horizontal starch gel techniques. The individual homogenates of 18 insects are run on a single gel. Following electrophoresis, the gel is sliced into 3 thin slabs for staining. In this way each insect is assayed for 3 different enzyme systems. If the homogenates are halved and placed on separate gels, 6 enzyme systems may be assayed. The assay procedures are being adapted from formulations previously used for other animal species. Genetic variation is visualized by the differential migration rates of allelic variants in the stained gel.

Results: Eighteen enzyme systems have been assayed in the caribfly. The preliminary results of this survey are presented in Table 1. No activity was observed for 1 enzyme (indicated by - in Table 1). Five enzyme loci (+) are monomorphic, i. e., they do not exhibit electrophoretic mobility differences between individuals. Electrophoretic variation for which a genetic basis has not been determined has been found in 6 enzyme systems (++) . Four systems are genetically polymorphic (+++).

Plans: Patterns of geographic variation in allele frequencies at several of the loci above will be studied. Comparative studies of the introduced Fla. population and endemic Caribbean populations should provide information on the "genetic structure" of a highly successful colonizer.



Table 1.--Survey of Protein Polymorphisms in the Caribbean Fruit Fly

Enzyme	Abbr.	Adult	Larva
Acid phosphatase	ACPH	++	++
Alkaline phosphatase	APH	≠ ACPH	= ACPH
Esterase	EST	++	+++
Leucine aminopeptidase	LAP	++	++
Alcohol dehydrogenase	ADH	+++	
Galactose dehydrogenase	GDH	--	
Glucose-6-phosphate dehydrogenase	G-6 PD	++	++
$\alpha$ -Glucose phosphate dehydrogenase	$\alpha$ -GPD	+	
Hexanol dehydrogenase	HexDH	+	
Isocitrate dehydrogenase	IDH	++	++
Malate dehydrogenase	MDH	++	++
Malate dehydrogenase	MDH-T	++	++
Octanol dehydrogenase	ODH	= HexDH	
6-Phosphogluconate dehydrogenase	6-PGD	+	.
Sorbitol dehydrogenase	SorDH	+	+
Adenylate kinase	AK	+	+
Hexokinase	HEX	+++	+++
Phosphoglucose isomerase	PGI	+++	





The White Peach Scale (Pseudaulacaspis pentagona) and its  
Natural Enemies: A Literature Review

T. R. Ashley and M. Winegar

Objectives: To determine what research has already been done on the white peach scale and its natural enemies. Once this goal has been reached it will be possible to determine the research which needs to be carried out in order to integrate the control of the scale into a pest management program for peaches making maximum use of nonchemical methods.

Methods: In compiling this literature review the following sources are being used: 1) the biological abstracts and card catalogues located in Hume and DPI libraries, 2) bibliographies from papers about the scale or its natural enemies, 3) local scientists who have worked with this scale or its natural enemies, and 4) the Current Awareness Literature Service.

Results: This literature review indicates that most of the research on the white peach scale has been in the areas of its history, distribution, ecology, number of hosts, morphology, genetics, and a listing of its natural enemies. The review also shows a number of areas which need a great deal more work. These include: 1) the role of the scale's natural enemies in the regulation of its populations, 2) the distribution of both the scale and its natural enemy populations in peach orchards of various ages, locations, and under different management programs, 3) the establishment of which natural enemies are regulating the scale on its natural hosts and why the scale is only considered to be a pest on peaches, and 4) the use of pheromones to either monitor an infestation level or disrupt successful reproduction. This review has also shown that the scale is only a pest on peaches which is a very good indication that 1 of 2 things is occurring. Either the natural enemies are not attracted to the peach tree or the present spray program is destroying them.

Plans: Once this literature review has been completed the next objective will be to survey the peach growing areas in the state of Florida to determine distribution, abundance, and species of natural enemies present in orchards of different ages, locations, and under different management systems. Cooperative research is planned to isolate, identify, and synthesize the scale's pheromone. We also plan to investigate the possibility of using this pheromone as a tool to either determine infestation levels or disrupt successful reproduction.



Parasites of the Housefly, Little Housefly  
and Soldier Fly in Poultry Houses

E. R. Mitchell

Objective: Determine the species of parasites associated with houseflies (Musca domestica), little houseflies (Fannia sp.) and soldier flies (Hermetia illucens) breeding in poultry manure.

Methods: Samples of poultry manure and sand (1/2 liter of each) were collected at weekly intervals beginning in April and continuing through the present from several commercial egg farms near Lake Butler, Fla. Housefly, little housefly, and soldier fly pupae were collected and held in the laboratory for emergence of parasites.

Results: Parasites recovered from fly pupae are shown in Table 1.

Plans: Survey will be continued.

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Table 1. Parasitic Hymenoptera reared from fly pupae, Lake Butler, Fla. 1973.

Parasitic Species	Host Species		
	Housefly	Little house- fly	Soldier fly
Pteromalidae			
<u>Mucidifurax raptor</u>	X	X	
Spalangidae			
<u>Spalangia cameroni</u>	X	X	
<u>Spalangia endius</u>	X	X	
Diapriidae			
<u>Trichopria</u> sp. or			
<u>Phaenopria</u> sp.			X



Effects of Hermetia illucens (L.) on Poultry Manure

F. C. Tingle and E. R. Mitchell

Objectives: The presence of the soldier fly, Hermetia illucens (L.) in poultry manure appears to affect both the accumulation of manure and the breeding of houseflies. Studies were initiated to determine the possibility of rearing H. illucens in the laboratory and/or holding the immature stages at low temperatures for release at later dates.

Methods: Hermetia illucens eggs were collected from poultry manure, placed on CSMA larval fly medium, and held in the laboratory at ca. 85°F. Pupae also were collected and held at selected temperatures of 45°, 55°, 65° and 82°F. After 2, 4, or 6 weeks at the 3 lower temperatures, the pupae were transferred to a cabinet maintained at 82°F where they were held for 4 more weeks. Adult emergence and emergence of parasites were recorded 1-3 times/week, and the sex ratio of H. illucens adults was determined.

Results: Rearing of H. illucens from eggs collected in the wild has been accomplished in the laboratory. Mating and oviposition have been observed in large field cages.

When field collected pupae were held at 45°F, no adults emerged. At 55°F, 27% emerged and at 65°F, 51% emerged. When held at 82°F, 48% emerged, all within the 1st 2 weeks of the 4-week period.

Table 1 shows the percent emergence of pupae as adults at 82°F after first being exposed at the indicated temperatures for 2, 4, or 6 weeks. It appears that field collected pupae could be stored effectively at 55°F for a period not exceeding 4 weeks.

The sex ratio of adults emerging from pupae in this study was 1.3 ♂: 1.0 ♀. The % of pupae with parasites (species not identified) emerging from them was 23% at 82°F (Table 1). The numbers of parasites emerging from 50 pupae were counted and was found to average 86 parasites/pupae.

Plans: Attempts will be made to rear H. illucens through its complete life cycle in the laboratory. Also, its specific effects on poultry manure will be investigated, including competition studies between H. illucens and Musca domestica. Effect of low temperature on the larval stages will also be investigated.



Table 1.--Emergence of adult soldier flies (SF) and parasites from soldier fly pupae collected from chicken manure. May 10, 1973.

Storage temp. Holding °F      period (wk.)		Adult SF(%) after 4 wk. at 82°F	% with parasites emerging after 4 wk. at 82°F
45	2	2	0
	4	0	0
	6	0	0
55	2	47	8
	4	34	0
	6	2	0
65	2	29	18
	4	0	18
	6	0	12
82	0	48	23





## Battery Operated Blacklight Traps

E. W. Hamilton

Objective: Blacklight traps are a valuable means of sampling many insect populations. However, blacklight traps are generally limited to AC operation and must be placed within a reasonable distance of an 115 VAC power source. Battery operated blacklight traps can be placed anywhere in the field for desired operation. Efficient and economical battery source power supplies are needed for blacklight operation.

Methods: Several means of converting 6 or 12 VDC, or even lower battery voltage sources, to a higher voltage for operation of 4 to 20 watt blacklights are being investigated. The lowest current drain consistent with satisfactory blacklight operation is being determined by actual field tests of completed systems.

Results: Blacklight power supplies operating from 1.5 VDC have been designed and are being tested in the field. The light emitted by the blacklight in this system is of low intensity but appears to attract a number of different arthropods. Several power supply systems operated from 6 or 12 VDC have been constructed. It is apparent that blacklight intensity with these systems depends in large part upon the amount of current demanded by the power supply in use. It has been discovered that up to 4 blacklights can operate from the same battery power supply when it is properly designed. No increase in current demand has been noted under multiple blacklight operation conditions.

Plans: Continue improving power supply designs for blacklight operation and investigate the required light intensity of blacklight sources that will attract various insect species. Decreased blacklight intensity requirements will allow lower battery power drain and longer operating periods from a given battery charge.



## Battery Operated Electric Grid Trap Power Supplies

J. M. Stanley, J. C. Webb, E. W. Hamilton,  
E. R. Mitchell and C. Masuda

Objectives: The battery operated high-voltage grid trap power supplies presently in use demand high battery current drain. Consequently, only 2 or 3 nights of operation are possible from one battery charge. A battery operated high-voltage grid trap power supply requiring minimal amounts of current is desired in order to reduce the amount of battery drain and extend field operation battery life.

Methods: Several methods of supplying high-voltage grid trap power are being investigated. Automobile ignition coils with a capacitor discharge system, photoflash ignition coils with a capacitor discharge system, and a handwound 12 VDC to 3600 VAC transformer converter system were designed and tested in the laboratory and the field. The converter system is similar to the Bodine unit described in another report.

Results: All of the systems operated at current battery drain of 100 to 750 ma, adjustable by an input resistor control circuit. These current demands were considerably below that required (3.5 amps) by the battery to AC converter units presently in use. Laboratory tests demonstrated that the insect knockdown capability of electric grid traps operated with these power supplies was comparable to the DC-AC inverter units. However, under field conditions, grid traps operated from the test power supplies were not as effective as the DC-AC inverter units. The transformer converter system is the simplest and appears to be the most promising of the new power supply designs.

Plans: Continued efforts will be directed at improving the direct DC-AC converter system for more effective operation. The effect of the power supply and grid trap electrical radiations on insects will also be investigated. Other means of prolonging battery life will be tested in relation to the efficiency of the grid trap operation: pulsed power supply operation; automatic means of power supply turn-off during the hours trap operation is not desired; and insect turn-on of the high-voltage killing system.



## Transformer Size for Electric Grid Traps

J. M. Stanley, C. Masuda, E. R. Mitchell and J. C. Webb

Objective: The level of electric current used on electric grid traps is critical in safety considerations. The safe let-go currents for women and men are 6 and 9 ma, respectively. A test was conducted comparing the insect trapping effectiveness of transformers with secondary currents of 30, 10 and 4 ma.

Methods: Three 12" diameter cylindrical grids with 1/2" electrode spacing were used in testing the three transformers - one transformer per grid. All were operated on a line perpendicular to the prevailing wind and baited with cabbage looper sex pheromone and virgin female tobacco hornworm moths. Traps were rotated daily.

Results: Three species of moths were collected - male cabbage loopers, soybean loopers and tobacco hornworms. There were no significant differences within any of the three species of moths of catches in grids powered by the three transformers with the different current ratings.

Plans: Grids will be powered by transformers with the lower current rating, as this size gave equal results to the more powerful units and are much safer.



## Comparisons of Electric Grid Shapes for Cabbage Loopers

J. M. Stanley, J. C. Webb, E. R. Mitchell and C. Masuda

Objective: Cylindrical electric grids have been very effective in evaluating insect pheromones and in determining insect populations. The construction of the grids has caused some difficulty because of the complexity of the design; thus, an effort was made to determine if a simpler design would provide comparable results.

Methods: Experimental grids were made with flat surfaces. All of these had 1/2" spacing between electrodes. The following units were field tested: (1) A flat grid ca. 17" high by 13" wide, (2) an angular grid assembled from two flat grids placed together to form a 60° vertical angle, and (3) a triangular grid assembled from three flat grids. Tests were made in the Hastings, Florida, cabbage production area during the spring using the cylindrical grid as a standard of comparison. Grids were spaced about 100 feet apart on a line that was as near perpendicular to the prevailing wind direction as the sites would permit and not interfere with the farmer's operations. Traps were rotated on a two or three night schedule. All grids were baited with synthetic sex pheromone which was placed on the flat and the angular grids so as to be on the "upwind" side of the prevailing wind. All grids were placed on 24" diameter funnel traps and operated with 4 ma, 120 volt a.c. transformers.

Results: The trap with cylindrical grid caught more but not always significantly more moths than the traps with the flat, angular or triangular grids. The trap with the triangular grid, which completely surrounded the pheromone as did the cylindrical grid caught more moths than either the flat or angular units. While the grids with the flat surfaces did not appear to be as effective as the cylindrical unit, these more easily constructed units might be effective for some research applications where a simple "yes" or "no" answer is satisfactory.

Plans: The cylindrical grid appears to be the most effective. We do not plan to test these grids further; however, if there is evidence later that more work is needed along these lines, the studies will be renewed.





Comparison of Electric Grid Trap Sizes and  
Electrode Spacing for Hornworms

J. M. Stanley, J. C. Webb, E. R. Mitchell and C. Masuda

Objectives: Cylindrical electric grid traps are very effective in the evaluation of pheromones of some insects. Preliminary to studies of the tobacco hornworm sex pheromone, an effort has been made to use the grids to study the attraction of males to virgin female moths and to determine the most satisfactory size grid and electrode spacing for these large moths. Some observations have been made that these moths are able to move vertically through the cylinder formed by the grid without being caught; therefore, we also need to determine the frequency of this activity.

Methods: Five cylindrical grid designs are being used: two are 12 in. in diameter with the electrode spacing on one 1/4 in. and the other 1/2 in.; one is 18 in. in diameter with 1/2 in. electrode spacing; and two are 24 in. in diameter with the electrode spacing on one 1/4 in. and on the other 1/2 in. In order to determine the movement through the cylinder, two grids of each size are used, one covered with hardware cloth to prevent the passage of moths and the other uncovered. All the grids are elevated in the funnels so that their geometric centers have the same angular relationship with the edges of the trap funnels. Each grid is operated with a 4 ma 120 volt current limiting transformer. They are placed on a line perpendicular to the direction of the prevailing wind adjacent to a field on which tobacco was grown in 1972. Grids are baited with three virgin female moths and traps are rotated daily.

Results: Male hornworm moth collections were started.

Plans: Tests will be continued through the current season and evaluated.



*Insect Attractants, Behavior, and Basic Biology Research Laboratory*

*USDA-ARS, Southern Region, Florida-Antilles Area  
1700 S. W. 23rd Drive at Archer Road*

*P. O. Box 14565  
Gainesville, Florida 32604  
904-373-6701  
FTS-373-5211*

*Derrell L. Chambers, Director*

Ecology Research Group

E. R. Mitchell, Research Entomologist; Ecology, pheromones (Res. Leader)  
W. W. Copeland, Agr. Research Technician  
R. H. Guy, Bio. Lab. Technician  
R. W. Hines, Agr. Research Technician  
C. A. Morgan, Biol. Aid.

T. R. Ashley, Entomologist\*; Ecology, biological control  
M. E. Winegar, Lab. Technologist I\*

C. O. Calkins, Research Entomologist; Ecology, population dynamics  
A. J. Hill, Agr. Research Technician  
M. McKoy, Agr. Research Technician  
V. A. Wicks, Lab. Helper\* (Part time, Temp.)

E. W. Hamilton, Research Entomologist; Bioengineering

M. D. Huettel, Entomologist\*; Genetics, behavior  
J. K. Young, Lab. Technologist I\*

N. C. Leppla, Research Entomologist; Biology, behavior, rearing  
I. D. Ainsworth, Lab. Helper\*  
S. L. Carlyle, Agr. Research Technician  
C. W. Green, Lab. Technician I\*  
W. J. Pons, Lab. Technician II\*  
L. W. Rodgers, Lab. Technician I\* (Temp.)

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A. Q. Antonio, Biol. Technician  
J. E. Brogdon, Lab. Technologist I\*  
M. I. Schmierer, Lab. Technician I\* (Part time, Temp.)

J. L. Sharp, Entomologist\*; Movement, distribution  
D. R. Bennett, Lab. Technologist I\*

F. C. Tingle, Research Entomologist; Ecology, attractants

\* University of Florida, Department of Entomology and Nematology, cooperating



### Biology Research Group

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W. G. Sercey, Biol. Lab. Technician
- J. A. Coffelt, Research Entomologist; Biology, pheromones  
R. K. Janka, Lab. Technician I\* (Part time, Temp.)  
W. T. McClellan, Lab. Technician II\*
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W. L. Jula, Lab. Technician I\* (Part time, Temp.)
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J. C. Fish, Lab. Technician I\*  
S. E. Hickman, Lab. Technician I\* (Part time, Temp.)
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P. C. Drummond, Biol. Lab. Technician\*  
G. M. Thompson, Lab. Technician I\* (Part time, Temp.)

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- H. R. Agee, Research Entomologist; Audio physiology  
M. L. Parks, Lab. Technologist I\*
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T. C. Carlisle, Biol. Lab. Technician  
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K. S. Woodburn, Lab. Technician I\*
- M. S. Mayer, Research Entomologist; Physiology, pheromones  
J. D. James, Biol. Lab. Technician



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R. R. Heath, Chemist

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(Res. Leader)  
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INSECT ATTRACTANTS, BEHAVIOR AND BASIC BIOLOGY RESEARCH LABORATORY

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*Address inquiries to:*

*D. L. Chambers, Director  
Insect Attractants, Behavior and  
Basic Biology Research Laboratory  
Agricultural Research Service, USDA  
P. O. Box 14565  
Gainesville, Florida 32604*

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# MANUSCRIPT STATUS REPORT

August 31, 1973

## NATIONAL TECHNICAL EDITOR REVIEW

- Calkins, C. O. and K. M. Kirk. Food preferences of Embaphion muricatum.
- Dutkowski, A. B. and H. Oberlander. Interactions between beta-ecdysone and fat body during wing disk development in vitro.
- Hagstrum, David W., James A. Coffelt and Claudia F. Tomblin. Influence of increasing or decreasing temperature upon oviposition of T. castaneum.
- Komarek, E. V., Betty B. Komarek, and Thelma C. Carlisle. The ecology of smoke particulates and charcoal residues from forest and grassland fires: A preliminary atlas.
- Leppla, Norman C., John L. Sharp, William K. Turner, Eugene W. Hamilton, and Dwight R. Bennett. Circadian rhythmicity of the adult lovebug, Plecia nearctica Hardy.
- Leppla, Norman C., Lonnie N. Standifer and Eric H. Erickson, Jr. Culturing blister beetle larvae on diets containing different kinds of honey bee collected pollen.
- Tumlinson, J. H., R. R. Heath and R. E. Doolittle. Application of chemical ionization mass spectroscopy to the determination of epoxide position in aliphatic chains.

## JOURNAL REVIEW

- Ashley, Tom R. and D. Gonzalez. Effect of various food substances on longevity and fecundity of Trichogramma. Submitted to Environ. Entomol. 6/6/73.
- Ashley, Tom R., D. Gonzalez and T. F. Leigh. Selection and hybridization of Trichogramma. Submitted to Environ. Entomol. 7/2/73.
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- Sower, L. L., K. W. Vick and K. A. Ball. Perception of olfactory stimuli that inhibit the responses of male moths to sex pheromones. Submitted to Science 6/5/73.
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